

# Discovering the Future: Modelling Quality Matters

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# Discovering the Future: Modelling Quality Matters

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Proefschrift  
ter verkrijging van de graad van doctor  
op gezag van de rector magnificus  
van Wageningen Universiteit,  
Prof. Dr. Ir. L. Speelman,  
in het openbaar te verdedigen  
op 26 Mei 2004  
des namiddags om 13 uur 30 in de Aula.

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Discovering the Future:  
Modelling Quality Matters

PhD thesis of Wageningen University – With summary in Dutch

May 2004

ISBN 90-8504-017-5

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**1**

**General Introduction**

De gustibus et coloribus non est disputandum. Even the Romans knew already that different individuals perceive taste and colour quite differently, and that one tries in vein to persuade somebody else of ones own perception and preferences. Over the centuries we have learned to live with that difference in perception and liking. In fact those differences between people provided, amongst others, the driving force for the development in abilities and variation that is so essential for mankind.

However, in modern society with the large spatial distance between the region of food production and the region of food consumption, production facilities and processing lines need some guidance and rules to accommodate large groups of consumers with one and the same product. To stay in the market, to stay competitive, it is no longer sufficient just to produce some food, no matter how good the taste and flavour is. The wishes, preferences and buying behaviour of consumers have to be taken into account. Local markets are vanishing rapidly, globalisation is the magic strategy.

So, for both the more traditional production and the anticipated future production systems of our food, science and practice are in desperate need of a good, workable and reliable definition and theory of quality. Good signifies that the theory must comprise all (at least the majority) of possible cases, workable means that it should be possible to acquire all information necessary to apply the theory, reliable indicates that scientists should understand the processes underpinning the quality related behaviour of consumers, and the associated behaviour of quality attributes and product properties.

All three accounts can be fulfilled by the power of good and fundamental modelling on product properties, quality attributes and consumer behaviour. The term fundamental is in this context somewhat ambiguous and prone to misunderstanding. A simple term that fully describes the systematic approach applied throughout this thesis does not exist. What is meant with fundamental (or more fundamental) modelling, as opposed to empirical modelling, is the development of models oriented towards processes occurring in the produce and based more on theoretical considerations than on available data, applying consistently the rules of a discipline and the laws of nature.

In this study, which a compilation of already published work conducted over the past 35 years of which the last 15 years were entirely devoted to the understanding and modelling of quality of agricultural products, it will be shown how reliable models can be built on theoretical considerations and calibrated against experimental results, while at the same time developing some understanding of and a good view on the ever-elusive food quality. Gradually over the long years of modelling processes, analysing experimental data and publishing obtained results, a workable translation towards practice and application emerges.

The generation of quality in the growing systems is brought to the front. The definitions of quality and quality attributes in the growing period are quite different from those in the postharvest chain. A strong effort is needed to bring those two worlds together, to ensure sufficient feeling with the changing demands of modern society. If we really want to understand quality of agricultural products, and if we really want to fulfil future consumer's demands, the generation of quality during the growing period has to be studied, unravelled and modelled. Even during the growing period, quality should be expressed in terms of attributes important to the consumer instead of important to the grower. This subject will not be a part of this work, but indications have already been collected that it should be possible to apply the same (or similar) lines of reasoning during the production of quality as well (Tijssens et al. 2002, 2003).

In Part 1 and 2 a more theoretical review will be provided on both theories and techniques. In all other parts of this work, examples are provided of applying these techniques and theories to practical experimental results. In most cases the data were obtained by other researchers, in search of information quite often different as finally found in the data, and the data were obtained before a theory or viewpoint was even developed. The main emphasis is therefore not to be put on the product or process at hand, however interesting in itself, since they constitute only the examples of the applied techniques. The main issue followed and chased



throughout this work is what can be achieved by proper and consistent application of a theory on quality and process oriented modelling.

The first Part is devoted entirely to the framework necessary for the development of reliable and reusable models. The system applied and advocated is primarily based on thorough decomposition of the problem at hand into constituting processes, a process oriented approach of modelling rather than a phenomenon oriented approach and a consistent application of fundamental rules of a particular discipline and the laws of nature, as far as they are known and understood. Being a chemist by education, the most appropriate discipline to choose was evidently kinetic modelling. The technique itself, its benefits and the consequences for practical application are highlighted based on a couple of papers and lectures.

Problem decomposition is a technique already known and applied for a long time in the area of artificial intelligence and information technology. The rules and application of problem decomposition itself, redirected towards application in the food area, are described in detail by Sloof (2001). The laws of nature and the fundamental rules of chemical and enzyme kinetics can be found in any good textbook on physics and chemistry (e.g. Chang 1984, Fersht 1984, Glasstone 1960, Godfrey 1996, Whitaker 1994). Some more details for application in the food area are highlighted by Van Boekel and Tijssens (2001).

Throughout all examples provided, the effect of temperature is described by Arrhenius law. In fact Van Boekel (2001) states: *Deviations of Arrhenius or Eyring's relationship are indeed possible, but very unlikely. It probably indicates that another reaction influences the one under study, and that problem decomposition is conducted improperly.* For practice that means that if a reaction rate constant is found in data analysis not to behave according to Arrhenius law, some neglected process is more important than anticipated.

Another very basic and fundamental effect is the way pH can and will affect reactions under study. Since these effects in literature are mostly approached and analysed with black box polynomial relations, a specific and fundamental approach to incorporating pH effects is described in more detail.

The second Part deals with a theoretical view on the nature of quality and the development of an applicable definition of quality, as perceived by users and consumers. It is built up and developed to be applied in fundamental and generic modelling of quality and quality behaviour of food and non-food agricultural products. The bottom line of the developed theory in the framework of modelling, is that the decomposition of quality into an intrinsic product quality, solely based on product properties and an assigned quality as perceived solely by consumers, does permit to describe the intrinsic quality of a product almost independent of consumer perception and preference. Also, directly emerging from this theoretical view on quality perception and intrinsic product quality is the acceptability of products by users and consumers. Acceptability is a combination of the intrinsic quality, and the assigned quality for a certain particular purpose. What is usually meant by product quality is in reality more often than not the acceptability of a product for a certain particular purpose.

In Part 3 some actual examples of models for the quality behaviour in postharvest storage, and transport are given, which include the omnipresent and ever-important enzyme and enzymatic behaviour, respiration and chilling injury.

In Part 4 some examples are provided that cover the processing of food products to ensure a longer product life, to adapt certain properties to consumer demands, or simply to avoid spoilage. With the first example (BRAM: Blanching Response Amplification Model) it is shown how simple and easy it is to combine developed models dedicated to only one or two separate processes into a more complex application. The behaviour of textural properties of vegetables is linked to a combination of the action of two denaturing enzymes during blanching with the physical destruction of firmness generating compounds during successive blanching and cooking treatments.

A very important aspect of both fresh as processed foods is microbial safety. Although not covered in this work in detail, it should be emphasised that the same fundamental line of reasoning, based on occurring processes rather than observed phenomena can successfully be applied in this realm. The well known square relation with temperature of the maximum growth rate, as reported by Zwietering (1993) and Wijtzes (1996)

$$\mu = b^2 \cdot (T - T_{\min})^2 \cdot \left(1 - e^{c \cdot (T - T_{\max})}\right)^2 \quad \text{eq. 1}$$

can very well be approached by the apparent rate of enzymatic reactions as developed in chapter 7:

$$k_{app} = k_s \cdot E_n \cdot e^{-k_d \cdot t} \quad \text{eq. 2}$$

for the activity of an enzyme  $E_n$  converting substrate with a rate constant  $k_s$  while denaturing at the same time with increasing temperatures with rate constant  $k_d$ . In Figure 1-1 an example is given for both relations, showing the good agreement between both approaches, considering the (probably) erroneous assumptions made in the polynomial analysis.

Part 5 deals primarily with the keeping quality or shelf life of perishable products, both in static and in dynamic conditions. The principles of keeping quality are easier to apply in more general terms in food distribution and food logistics (Broekmeulen 2001) as a kind of substitute for quality expressed in predefined specifications. The preferences for different groups of consumers in terms of quality limits of acceptance are incorporated into this extended approach of the empirical shelf life system already known for almost a century.

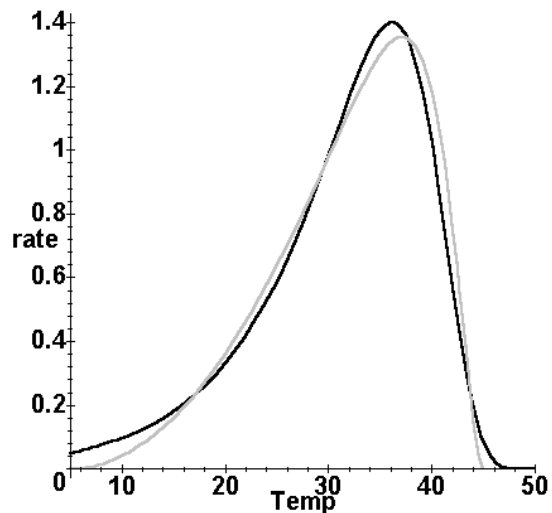


Figure 1-1 Comparison in behaviour of bacterial maximal growth rate  $\mu$  (light line), and the more theoretical rate constant of a denaturing enzyme (dark line).

In Part 6, the emphasis is put on the variability that is almost invariably observed in batches of product. Biological variance is generated by whatever reason during the growth and harvest of agricultural produce. Variance in batches of produce is always present. It cannot be avoided completely, but the magnitude of variance can only be diminished to a manageable level, by e.g. sorting and grading and targeted growing. In this part is described and modelled how variance, once present in the batches of product will develop during storage and transport through the ripening of the product. Not only the behaviour of quality, but also the behaviour of the associated biological variance is strongly connected and defined by the type of mechanism involved and the magnitude and rate of the changes.

Finally in Part 7, it is shown that food research, food quality modelling and quality of scientific research can be expressed in very simple terms that can be understood by experts and layman alike.

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## **Part 1**

# **PARADIGM OF MODELLING, OLD RULES, NEW APPLICATIONS**

## Introduction

The ultimate goal of modelling is to provide reliable predictions of occurrences that did not yet take place. In the formulation of Rickert (2001): *Models can be regarded as a repository for past research since they collate and integrate information from past research.* This goal, although agreed upon by every modeller and every user of models, is at the present time, however, far out of reach and far away from being fulfilled by the present technology in modelling land. Oh yes, modellers have achieved major progress in mathematics, modelling techniques and modelling tricks during the past few decades, especially in the modelling of crop production systems, from the early sixties of past century (Thornley 1976, 1990) up to the 21<sup>st</sup> century (Lee 2002). What technology can deliver at the moment, is a fairly accurate description of the behaviour of a product or commodity in conditions not too far away from the conditions at which the results have been gathered to validated or calibrated the model.

For modelling the quality behaviour of our food from the growing site through the different handling sequences, the distribution chain, storage, processing up to consumption and the final judgement of consumers, that situation is not different. A vast number of models, submodels and applications have been built and some of them have been reported in detail. Most of these models are very valuable for exactly that but only that application: predicting the future behaviour of a product, in circumstances similar to the test circumstances. These are the so-called dedicated models. From the moment on, the application is extended to fairly new circumstances the sometimes huge task of developing, creating and calibrating new models has to be taken up again. In terms of efficiency of resource utilisation, this approach seems efficient in the short term, in the long run however, this approach is not very efficient or satisfying at all. Moreover, in terms of understanding the problem at hand and in terms of generating knowledge about the problem at hand, which is the ultimate goal of all science, this empirical approach lacks inherently any value, since they are completely based on the mathematical description of phenomenological observations.

To achieve the ultimate goal of modelling: predicting future behaviour in any circumstance, from any region, grown in any season while generating more knowledge about the process under study, we need to and have to include all available fundamental knowledge that is at our disposal. The results of this kind of approach are the so-called fundamental (or rather more fundamental, process describing) models. These do indeed generate more knowledge and understanding, and can direct scientific research to those areas where information and understanding seems to be lacking.

Two nice examples of very successful models or thinking systems based on practical principles already applied for a long time without virtually any fundament in theory, are keeping quality and the often-used temperature sum. The first example will be handled in full in chapter 13 (keeping quality), the latter example can be found in Tijssens and Verdenius (2000).

Fundamental oriented models can be built in many ways and within many scientific disciplines. Disciplines, however, are a result of the deep-rooted urge of man and scientists to bring some order in the view and perception of the world. Nature does not have nor need disciplines at all. Processes occurring in nature comply “naturally” with the laws of nature. The ultimate drive of science is to detect, reveal and understand those laws, and to convert the understanding of these laws into some practical application to increase the quality of our lives. So, within the realm of one discipline, the same laws of nature, known and recognised in other disciplines will be present and known in one form or another. The direct consequence is that in dealing with processes of nature and modelling them, it does not really matter which particular discipline is used as a framework, as long as its rules are used consistently and thoroughly. Being a chemist by education, and since many processes in our food products are of chemical nature, the choice of kinetic modelling was self-evident. The general rules of kinetic modelling are summarised and described in van Boekel and Tijssens (2001).

The rules of discipline and the laws of nature in the area of chemistry were all discovered and formulated in the early days of chemical discoveries. The rules of chemical kinetics were formulated about 100 years ago as can be found in good old-fashioned textbooks of physical chemistry like Glasstone (1960). Theories on thermodynamics, temperature dependence, ionic dissociation of water and the associated pH and ionic strength were formulated and are now a standard part of textbooks on chemistry, physical chemistry, kinetics and enzyme kinetics (e.g. Chang 1984, Fersht 1984, Glasstone 1960, Whitaker 1994).

Although this knowledge is available for so long a time, when building old-fashioned empirical models, this knowledge is the first information that consistently is not used at all. Every time new data need to be analysed and interpreted, mathematical and statistical relations are invented and tested, and the model with the least number of parameters and with the statistical best fit to the data at hand is chosen to represent the behaviour of that system, without including any expert knowledge whatsoever. Modern science is based on the most important rule of all: the repeatability of a process. That is: in the same conditions the same process will occur with the same rate. In making models, we should use this very basis of science and search for process rate constants that are true constants for any condition encountered. In other words in kinetic modelling, the observed rates of processes may depend on the actual conditions, the rates constants of processes, however, will be the same for the same processes, no matter what the conditions are.

Nevertheless, some general rules can be drawn up when modelling the complexity of interacting processes that occur in nature. When targeting at modelling interactive processes, it is of utmost importance to apply the old Roman rule "divide et impera" (divide and rule). In modern terminology that is problem decomposition. Having used this technique almost unconsciously for several years in developing process oriented models, encountering numerous difficulties understanding ones own actions, a young information technologist devoted a major part of his PhD thesis to develop a more consistent, understandable and applicable system in that respect (Sloof 1999, Sloof 2001). Still, the process of detecting possible and plausible mechanisms in rather unstructured data is apparently quite a difficult task for most people. In my opinion, that ability is exactly what makes a modeller a good modeller, rather than the necessary requirements of mathematical skills, statistical skills or product expertise.

In chapter 2 the consequences of fundamental and kinetic modelling for practical applications is worked out in more detail, based on a number of modelling and application examples. In this chapter the consequences and the power of process-oriented models is clearly deduced and indicated.

Another general rule for developing process-oriented models is the consistent application of the laws of a particular discipline at all cost. Adapting mathematical equations just for the ease of the resulting equation, whether at the level of differential equations or at the level of functions or analytical solutions of these differential equations, invariable changes the fundamental nature of a model into an empirical one. Changes at a mathematical level inevitably prevent any further development along fundamental lines.

Not only process oriented modelling offers the advantage of an increased understanding of processes and interactions, it also ensures the reusability of models and above all the transferability of parameter values to all kinds of different situations and conditions the product is used at.

How modern fundamental modelling really is, can be taken from the fact that all fundamental knowledge used, especially the laws of nature and the rules of a particular discipline, were discovered and formulated about 100 years ago. Examples of these are the Arrhenius (1889) law and Eyring law (1965) for the dependence of rate constant on temperature, and the application of the theory of dissociation of water and the connected theory on acids, bases and pH that can be found in all good textbooks on (enzyme) kinetics and physical chemistry (Fersht 1984, Chang 1981, Whittaker 1994 etc). Both theories were applied with astonishing success in describing exactly that what they claim. In chapter 3 the advantages and consequences of fundamental process-oriented modelling regarding pH effects is indicated.

The possible consequences for application in research and practice are worked out in more detail to elucidate the power temperature and pH to describe fully the behaviour of various chemical and enzymatic systems up till the behaviour of complete horticultural systems. More examples of this line of reasoning for describing pH effects in product behaviour can be found in Tijskens et al. (2001a), Tijskens and Biekman (2001), Tijskens et al. (2001b) and Seyhan et al. (2002).

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**2**

**Generic modelling and practical applications**

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Based on a lecture held at the combined COST 915 & COPERNICUS/CIPA Symposium, Food Quality Modelling. June 4-6, 1997, Leuven, Belgium. Proceedings p. 145-151 (*Food Quality Modelling* B.M. Nicolaï, J. de Baerdemaeker eds.), 1998.

## Introduction

Nowadays many models are built in food research. The subjects of these models cover a vast field of products, preharvest and postharvest treatments, including thermal food processing, storage and microbiological safety. Most models are primarily aimed at obtaining knowledge about the complex effects of the applied treatments. As such, they constitute a major improvement for research efficiency, research efficacy and data analysis. Applications in practice, however, for commercial and trading purposes are still quite limited to local fields and to models dedicated to one treatment or treatment condition.

Based on the experience in building models in the field of enzymatic action and enzyme denaturation by heat, development and decay of quality attributes (like firmness and colour) during storage and processing for a large range of fruits and vegetables (tomato, apple, peach, carrot, mango, mushroom, potato), a viewpoint has been developed that allows to build models that describe the observed quality changes in a generic fashion. How to arrive at useful and practical formulations for models in physiology is already described by Tijssens & Sloof (1996). In this chapter we will concentrate on the significance and implications of generic modelling for research and practical applications.

Regardless of the biological variance induced by numerous conditions like weather, fertilisation, rainfall etc., the processes acting on quality defining properties are common for all batches. It is very unlikely that enzyme systems will act completely different in different batches (from different seasons or regions) of the same species/cultivar. The different behaviour of these batches of product of the same cultivar, could be ascribed to different levels of initially present substrates and enzymes. This inherently signifies that, provided the model is based on a correct reflection of the processes occurring in the products, the model parameters can be ranked into a group common for the cultivar (kinetic parameters like reaction rate constants, activation energies) and a group specific for a batch (like maturity, initial firmness and colour). As a consequence, the common kinetic parameters can (more) easily be determined on a laboratory scale and transferred without conversion to practical applications. The batch specific parameters have to be estimated or determined for each individual batch under consideration.

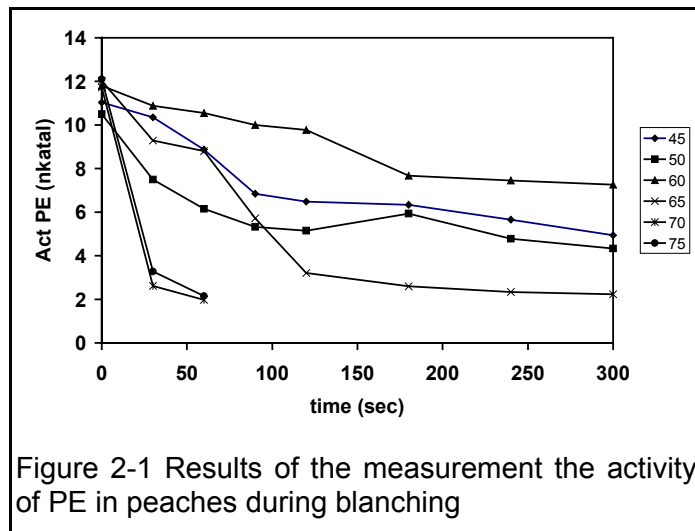


Figure 2-1 Results of the measurement the activity of PE in peaches during blanching

## Phenomenon oriented modelling

Mathematical models can be developed that describe the behaviour of some property or attribute in a set of data gathered within one experiment or a group of similar experiments. A classical approach is to analyse data for each temperature separately.

Establishing the decrease in activity of the enzyme pectin methyl esterase (PE) in peaches during blanching at different temperatures, it was found (Figure 2-1) that for each temperature separately, ranging from 45 to 75 °C, the decrease in firmness could be described very well with an exponential function, including a certain amount of remaining activity, as can be expected for a first order decay reaction what denaturation really is (see (1)):

$$\begin{aligned}
 & \text{PE} \xrightarrow{k_d} \text{PE}_{na} \\
 & \text{PE} = \text{PE}_{var} e^{-k_d t} + \text{PE}_{fix}
 \end{aligned}
 \tag{1}$$

Although the data of each of the separate temperatures fitted quite well the general exponential equation, the parameters showed major differences between the successive series (see Table 2-1). Roughly one can discern two levels in both parameters connected to activity ( $PE_{var}$  and  $PE_{fix}$ ). The rate constant  $k_d$  shows a behaviour that cannot be explained or described by the standard laws like Arrhenius law.

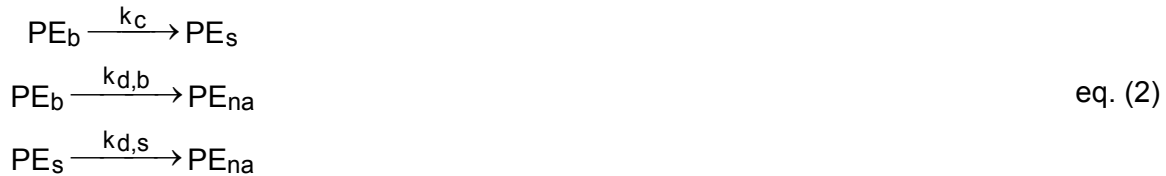
The consequence of this observation is that one cannot apply the results of this analysis and hence that one cannot apply this simple model in a much wider context of processing circumstances. Certainly not in dynamic conditions where both time and temperature change simultaneously. So, to increase the applicability of the model we have to dig in deeper in the relations and processes that exist undoubtedly in this system.

Table 2-1 Classical analysis of PE activity in peaches at each temperature separately

Temp [°C]	$k_d$ [1/sec]	$PE_{var}$ [nkatal /gFWT]	$PE_{fix}$ [nkatal /gFWT]	$N_{obs}$ [-]	$R^2_{adj}$ [-]
45	0.00951	6.62	4.75	8	93.8
50	0.02563	5.559	4.933	8	93.3
60	0.00353	7.427	4.412	8	94.1
65	0.0102	11.12	1.24	8	93.7
70	0.08246	9.929	1.8 (fixed)	3	100
75	0.06386	10.297	1.8 (fixed)	3	99.9

## Process oriented modelling

Without going into the details of determining the mechanism, the behaviour of the PE activity during blanching as a function of time and temperature could be described by a more complex mechanism as shown in scheme (2). A bound configuration is converted into a soluble one. Both species are susceptible to denaturation.



The total activity is described by the sum of both isoenzymes, including an invariable portion of the PE activity (eq. (3)):

$$PE_{tot} = PE_b + PE_s + PE_{fix} \tag{3}$$

With the equations, derived from this mechanism for constant conditions of temperature, together with the equation of Arrhenius to express the temperature dependence of reaction rate constants, the data were reanalysed. The results are shown in the first column of Table 2-3 (data 1994 season).

With this approach, all temperature series have been combined in the statistical analysis. As a result the model formulation and the associated model parameters obtain a much wider possible application: the effect of all temperatures within the measured range, can now be described and predicted. The three-dimensional representation of the activity of pectin methyl esterase versus time and temperature is shown in Figure 2-2.

## Including Batch and Seasonal effects

These data described were collected from peaches from the same origin and about the same harvest date. So, they were grown in comparable situations. The picture gets more complicated when batches from different growing areas (origin) or different climatic conditions (seasons) during growth are used in experiments, or in industrial processing. The same model for PE activity as function of temperature and time, could be applied to data gathered in the following season (1995). Although the behaviour looked quite different compared to the data set of 1994, and although the parameters estimated for the reaction rate constants were slightly different, (see second column Table 2-3) it was possible to analyse the data of both seasons combined with the kinetic parameters in common (except

for  $k_{d,s,ref}$ ), but with separate initial levels of  $Pe_b$ ,  $Pe_s$  and  $Pe_{fix}$  (see third column Table 2-3).

As a consequence, we can safely conclude that, provided the constructed model is based (as good as possible) on the processes that do (really?) occur in the product, the model parameters can be subdivided into batch dependant parameters (all initial levels) and kinetic parameters (all reaction rate constants and activation energies). As it is most of the time not known how different conditions (growing, season) affect the batch parameters, these parameters, that means all levels of initial concentrations (or amount) relevant for the model, have to be determined for each batch separately. As the kinetic parameters are supposed / proven to be specific for a cultivar, the kinetic parameters for that cultivar can be

determined once on laboratory scale, and applied in the future for each batch of that cultivar. The same reaction mechanism and the same model formulation have successfully been applied to pectin methyl esterase activity in potatoes and carrots (Tijssens et al. 1997e), and to a number of enzymes like PG (Tijssens et al. 1997b, 1997c), POD (Tijssens et al. 1997d), lipoxygenase (not published) and lipase (Ponne et al. 1996).

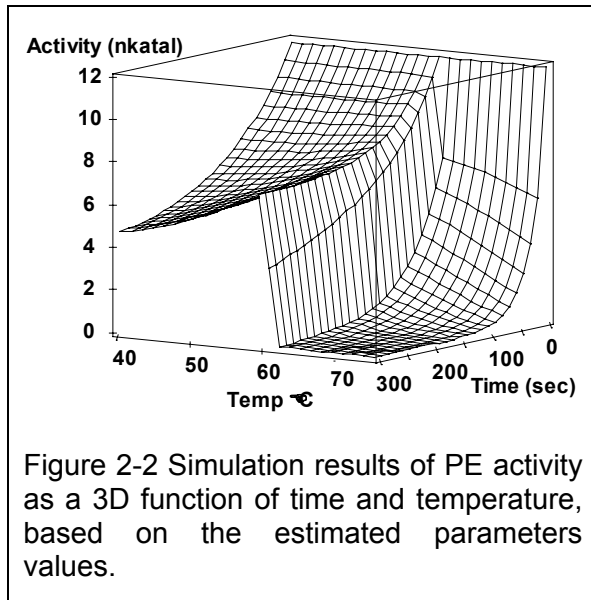


Figure 2-2 Simulation results of PE activity as a 3D function of time and temperature, based on the estimated parameters values.

### Transferability of model parameters

One of the consequences of the deduced system so far, is that model parameters should have identical or similar values when the processes, upon which they are relying or to which they are referring, are the same irrespective of the variables measured in a specific batch of products. If the firmness of some product is changing by the action of an enzyme, the kinetic parameters of that enzyme (like denaturation related parameters) should be the same in any (similar) experiment or technical process concerning heat processing of that product.

Within the same project as for the peaches, the activity of pectin methyl esterase and the firmness remaining after a combined blanching / cooking treatment was measured in carrots. Analysing the PE activity according to an extended model formulation, based on a mechanism similar to eq. (2), an explained part of 92.7% was found (see Table 2-2, column 1).

$$\text{Firm} = \text{Firm}_{\text{fix}} + \text{Firm}_0 \left( 1 - e^{-\frac{k_s PE_0 (e^{-kt} - 1)}{k_d}} \right) \quad \text{eq. 4}$$

The firmness of carrots from the same batch after cooking was measured and analysed according a simple semi-empirical equation (eq. (4)) and according an extended model based on first order decay of the firmness component by a first order denaturing enzyme (eq.(5)), developed as based on existing knowledge and expertise in the field of processes occurring during blanching and cooking.

$$\text{Firm} = \text{Firm}_{\text{fix}} + \text{Firm}_0 e^{-k_\beta t_c DE_0 e^{-k_d t_b - 1}} \quad \text{eq 5}$$

The results of the statistical non linear regression analysis for some of the relevant kinetic parameters are shown in Table 2-2 (column 2 and 3). We have to bear in mind that for both the analyses on carrot firmness, the estimated rate constants for the denaturation of PE is entirely based on firmness measurements. The estimated values according the simple firmness model do not show any resemblance with the results obtained in the direct activity

Table 2-3 Results of the nonlinear analysis of PE activity for two seasons separately and combined

Parameter	1994		1995		'94 + '95 combined	
	estimate	s.e.	estimate	s.e.	estimate	s.e.
<b>Kinetic parameters</b>						
$k_{c, \text{ref}}$	0.00749	-	0.0103	0.00797	0.01027	0.00393
$E_c/R$	9904	-	0	-	8031	2259
$k_{d,b, \text{ref}}$	0.008	Fixed	0.008	fixed	0.008	Fixed
$E_{d,b}/R$	500	Fixed	500	fixed	500	Fixed
$k_{d,s, \text{ref}, 1994}$	4.11E-05	-			3.91E-04	1.96E-04
$k_{d,s, \text{ref}, 1995}$			2.20E-08	8.00E-08	3.33E-07	5.44E-07
$E_{d,s}/R$	1117393	-	87861	26346	68143	10713
<b>Batch parameters</b>						
$PE_{\text{fix}, 1994}$	0	fixed			0	Fixed
$PE_{b, 45, 1994}$	8.573	-			9.2	1.93
$PE_{b, 50, 1994}$	6.118	-			6.74	1.86
$PE_{b, 60, 1994}$	10.14	-			10.81	1.54
$PE_{b, 65, 1994}$	9.266	-			9.94	1.63
$PE_{b, 70, 1994}$	8.468	-			8.73	1.57
$PE_{b, 75, 1994}$	9.484	-			9.99	1.66
$PE_{s, 0, 1994}$	2.872	-			2.24	1.46
$PE_{\text{fix}, 1995}$			0	fixed	0	Fixed
$PE_{b, 45, 1995}$			2.93	1.93	2.37	1.32
$PE_{b, 50, 1995}$			5.51	1.72	4.96	1.24
$PE_{b, 55, 1995}$			5.13	1.76	4.37	1.22
$PE_{b, 60, 1995}$			3.65	1.89	2.720	1.22
$PE_{b, 65, 1995}$			3.68	1.89	2.57	1.19
$PE_{b, 70, 1995}$			1.9	2.07	0.84	1.16
$PE_{b, 75, 1995}$			5.81	1.86	4.7	1.12
$PE_{b, 80, 1995}$			4.79	1.88	4.05	1.16
$PE_{s, 0, 1995}$			4.15	1.64	4.879	0.859
<b>Administrative information</b>						
$T_{\text{ref}} (^{\circ}\text{C})$	60		60		60	
$R^2_{\text{adj}, \text{tot}}$					91.5	
$R^2_{\text{adj}, 1994}$	92.3				92.8	
$R^2_{\text{adj}, 1995}$			88.1		90	
n	38		52		90	

analysis (see Table 2-2, column 1 and 2). However, with the extended model, having the same explaining power as the simplified model, the estimated parameters for the enzyme stability during blanching are almost exactly the same. From these results we can take that even when the model is much more complicated by the applied extension, the value and applicability of the parameters are of much greater importance than for a simplified model.

## Generic Modelling

Extending even more the applicability of mathematical models, one arrives at generic modelling. Up to now the batch and seasonal effects are included without any

Table 2-2 Results for the PE activity in carrots, based on activity measurements and firmness decrease during blanching and cooking

Parameter	Enzyme		Firmness
	Extended	Simple	Extended
$K_{d, \text{ref}}$	0.04407	0.01224	0.04871
$E_d$	19318	31998	19489
$K_s$	-	0.011299	0.24165
$E_s$	-	12439	13653
$R^2_{\text{adj}}$	92.7	93.1	95.4

interpretation of the meaning and the origin of the observed differences between batches and growing conditions. Models will become much more powerful for practice, when links between batch oriented parameters and the cause of difference can be unravelled or at least indicated.

In a study on reducing sugars in potatoes during storage at different constant temperatures, it was found (Hertog et al. 1997) that one model formulation could account for quite different apparent behaviour of cold induced and senescence induced sweetening. The analytical solution at constant temperatures of the constituting differential equations is shown in eq. (6). The kinetic parameters of the model could be estimated as specific for each cultivar (4 different cultivars) but in common over a number of seasons and origins (maximum number of three) for each of the cultivars.

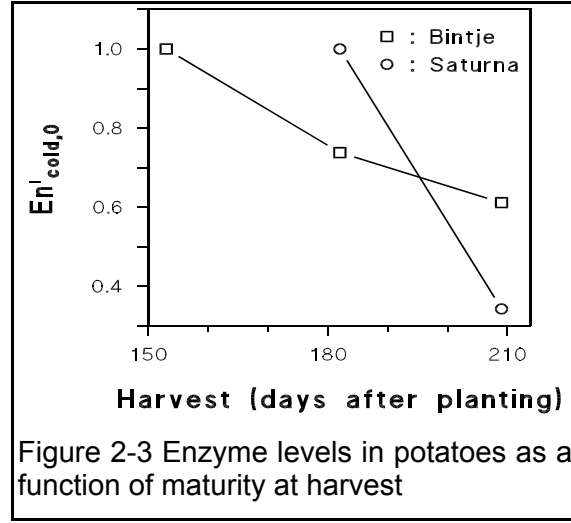


Figure 2-3 Enzyme levels in potatoes as a function of maturity at harvest

$$\begin{aligned}
 S = & \frac{k'_{cold} En'_{cold,0} (e^{-k_{dena} t} - e^{-k_{resp} O_2 t})}{k_{resp} O_2 - k_{dena}} \\
 & + \frac{k'_{scen} En'_{scen,0} (e^{-k_{form} t} - e^{-k_{resp} O_2 t})}{k_{resp} O_2 + k_{form}} \\
 & + S_0 e^{-k_{resp} O_2 t}
 \end{aligned}
 \tag{eq 6}$$

In the combined analysis, all seasonal effects were attributed to one unnamed enzyme. For two cultivars, it was observed that the level of that enzyme had a striking relation with the maturity at harvest, roughly estimated as the time after planting (see Figure 2-3). These results were further validated based on an independent set of data, were in one season, the effect of maturity on the sweetening of potatoes during subsequent storage was monitored. The same model formulation could be applied to this independent data set (see Figure 2-4). The effect of maturity, which was much better defined due to the more or less invariable seasonal influence, was again very well described by assuming that single unnamed enzyme to be the cause of different behaviour (see Figure 2-5).

The results of these experiments and analyses open a complete new road to practical applicability of fundamental models. Studies are now being conducted in search of enzymes

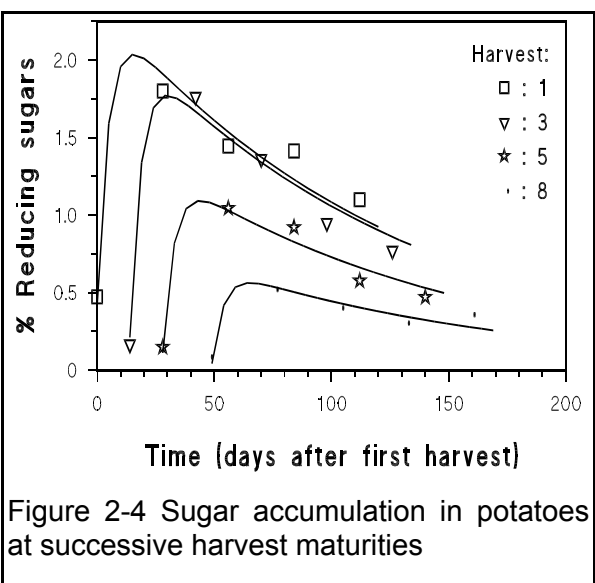


Figure 2-4 Sugar accumulation in potatoes at successive harvest maturities

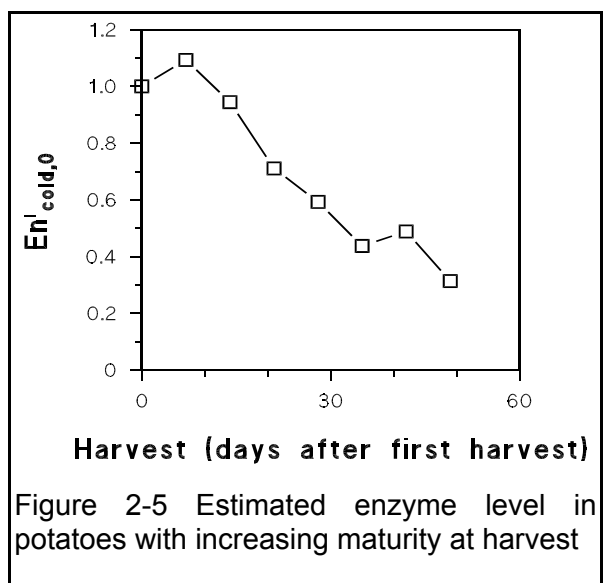


Figure 2-5 Estimated enzyme level in potatoes with increasing maturity at harvest

that match the predicted properties of this up to now unnamed enzyme, responsible for the maturity effects on cold induced sweetening in potatoes during storage. If (when) this enzyme will be found and determined, it will be possible to predict the type of and the susceptibility to cold induced sweetening in potatoes at the moment of harvest.

## Conclusions

Strong indications have been obtained that with correct model formulation, the estimated parameters can be transferred from one laboratory experiment to another one, from one batch of products to a next one. A direct consequence of this approach is the classification of model parameters to either be cultivar specific or batch specific. This will/may reduce research efforts to characterise batches at the start or in the food chain and increase research efficacy.

Another consequence is a tremendous improvement in scaling up from laboratory scale (in vitro) to practical scale (in vivo). By proper generic model construction and subsequent analysis, the vast knowledge of experienced researchers and the complete pool of (unused / not fully used) research results could be applied more efficiently and transferred to practical and commercial applications.

What remains to be done (largely) is to build models in a generic fashion as described, to conduct the transfer of knowledge from research to practice.

## Acknowledgement

The project on texture and enzyme kinetics of peaches and carrots was partly financed by the EU (project AIR1-CT92-0278), the project on sweetening of potatoes was partly supported by the Dutch Commodity Board for Potatoes.

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**3**

**Modelling the effect of  
temperature and pH on the activity of enzymes:  
the case of phytase**

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Published in *Biotechnology & Bioengineering* 2001, **72 (3)**, 323-330.

## Abstract

The behaviour of enzyme activity as a function of pH and temperature was modelled based on fundamental considerations. A formulation was developed that includes the activation of enzymes with increasing temperatures, the deactivation of enzymes at higher temperature, together with the effect of protonation and hydroxylation on the activity with various constant pH levels. The model was calibrated and validated on an extensive set of experimental data on phytases from seven different origins. The percentage variance accounted for ( $R^2_{adj}$ ) obtained by statistical non-linear regression analysis on the entire data sets, ranged from 97.6 to 99.5%. The equilibrium constant of protonation and hydroxylation was independent of temperature.

## Keywords:

Enzyme activity, pH, temperature, denaturation, activation, phytase.

## Introduction

Enzymes are important catalysts in all kind of living material. The mode of action of enzymes is not always clearly understood, let alone described and modelled. One of the difficulties for understanding the exerted action (the cumulative conversion catalysed by enzymes) is that enzyme activity depends on temperature and pH. Most of the time, empirical models are used to describe and predict the activity of enzymes as a function of temperature and pH. In all kinds of polynomial functions, pH is used as explaining variable. Matters get even more complicated when one tries to include the effect of temperature on the enzyme activity.

As such, these empirical models provide a useful description and mostly a fair prediction of enzyme activity in batches and situations that closely resemble the batch and the situation used in the experimental set-up. They lack, however, any bearing with the processes that occur in reality, and can therefore not be used to extend the specific knowledge and the specific application of enzymology. More fundamental models are necessary to understand more fully the behaviour of enzymes in all kinds of situations encountered in practice. Although these more fundamental oriented models are more reliable in general, they sometimes lack predictive power in specific situations. The explanation of this observation is that empirical models describe inherently all side effects as well, while fundamental models only describe the considered and included processes. However, they may allow prediction outside the range of experimental calibration, and contribute as such to the understanding of enzyme activity, and provide direction to further research.

In this paper, based on fundamental principles a model was developed to include the effects of pH and temperature on enzymes activity in general. The effects of a large range of temperature and pH, on the activity of the enzyme phytase from different origins were studied and the data were analysed based on the developed fundamental model. The principles applied are so general that they can easily be adapted and applied to other enzymes.

Phytases (*myo*-inositol hexakisphosphate phosphohydrolases) belong to a special group of phosphatases capable of sequentially hydrolysing phytate [*myo*-inositol(1,2,3,4,5,6) hexakisphosphate], the major storage form of phosphorus in seeds and pollen (Reddy et al. 1989). This results in a stepwise formation of *myo*-inositol pentakis-, tetrakis-, tris-, bis-, and monophosphates. In this sequential hydrolysis orthophosphate is liberated. Phytases are widely distributed in nature, for example in plants, certain animal tissues and microorganisms, particularly fungi. Different types of phytases are known: 3-phytases (EC 3.1.3.8), 4-phytases, and 6-phytases (EC 3.1.3.26), indicating the predominant attack of the susceptible phospho-ester bond. Phosphatases are widely distributed in nature and hydrolyse a broad spectrum of phosphate esters. Phytases have been studied intensively in the last few years because of the great interest in applying such enzymes to reduce phytate content in animal feed and food for human consumption. Phytase was originally proposed as an animal feed additive to enhance the value of plant material by liberating orthophosphate (Mitchell *et al.*

1997). More recently, phytase addition has been considered as a mean to reduce the level of phosphate pollution in areas of intensive livestock management (Cromwell et al. 1995). As phytate also can act as an anti-nutrient by chelating minerals, such as zinc, iron, calcium and magnesium, addition of phytase can improve the nutritional value of plant-based foods. It enhances the reduction of phytate during digestion in the stomach (Sandberg et al. 1996) or during food processes such as soaking, grinding, malting, fermentation, heat treatment, and germination (Reddy et al. 1982; Greiner and Jany 1996). Furthermore, phytases are of great interest in the production of special isomers of different lower phosphate esters of *myo*-inositol. Certain *myo*-inositol phosphates have been suggested to have positive effects on heart diseases by controlling hypercholesterolemia and atherosclerosis (Jariwalla et al., 1990; Potter, 1995) and to prevent renal stone formation (Modlin, 1980; Ohkawa et al., 1984). The most extensively studied positive aspect of *myo*-inositol phosphates is their potential for reducing the risk of colon cancer (Baten et al., 1989; Graf and Eaton, 1993; Shamsuddin et al., 1997; Ullah and Shamsuddin, 1990; Vucenic et al., 1993; Yang and Shamsuddin, 1995). Furthermore, much attention has been focused on lower *myo*-inositol phosphates, since some of these compounds, in particular D-*myo*-inositol(1,4,5) trisphosphate and D-*myo*-inositol(1,3,4,5) tetrakisphosphate, have been shown to play an important part as intracellular second messengers (Potter, 1990). Several isomers of *myo*-inositol phosphates have shown important pharmacological effects, such as prevention of diabetes complications (Carrington et al., 1993; Ruf et al., 1991) and anti-inflammatory effects (Claxon et al., 1990; Siren et al., 1991).

## Materials and Methods

### ***Enzymic Materials and Purification***

Phytases from seven different origins (five from grain species, two from bacterial species, see Table 3-1) were obtained. The purification of the phytate-degrading enzymes was performed as described previously (see Table 3-1). The specific phytase activity of each of the types used throughout the investigation is also provided in Table 3-1.

Table 3-1 Types of phytase and standard activity applied

Phytase type	Activity in U mg <sup>-1</sup>	Reference
<i>Escherichia coli</i> P2	8000	Greiner et al. 1993
<i>Klebsiella terrigena</i>	205	Greiner et al. 1997
Barley P1	115	Greiner et al. 2000
Barley P2	40	Greiner et al. 2000
Oat	307	Greiner and Larsson Alminger 1999
Rye	529	Greiner et al. 1998
Spelt D21	260	Konietzny et al. 1995

### ***Experimental set-up***

The activity of phytases from these seven different origins was determined at pH range from 1 to 9.5 in a .5 pH sequence, at temperatures from 10 up to 90 °C in steps of 5 °C. The following buffers were used: pH 1-3.5 glycine/HCl; pH 3.5-6 Na-acetate/H-acetate; pH 6-7 Tris/H-acetate; pH 7-9 Tris/HCl; pH 9-10 glycine/NaOH. The pH-values of the buffers were adjusted at the corresponding temperature.

The incubation mixtures for the determination of phytase activity consisted of 390 µl 0.1 M of one of the buffers, containing 350 nmol phytic acid dodecasodium salt (Aldrich, Steinheim, Germany) as substrate. The enzymatic reactions were started by addition of 10 ml enzyme to the assay mixtures. After an incubation period of 15 min, the liberated phosphate was quantified by a modification of the ammonium molybdate method (Heinonen and Lahti, 1981). Added to the assay mixtures were 1.5 ml of a freshly prepared solution of aceton:5 N sulphuric acid:10 mM ammonium molybdate (2:1:1 v/v) and thereafter 100 µl 1.0 M citric acid. Any cloudiness was removed by centrifugation prior to the measurement of absorbance at 355 nm. In order to

quantify the released phosphate a calibration curve was produced over the range of 5 to 600 nmol phosphate. The rate of reaction was linear for the 15 min incubation times (data not shown). Activity (U) was expressed as 1  $\mu$ mol phosphate liberated per min. Blanks were run by addition of the ammonium molybdate solution prior to addition of the enzyme solution to the assay mixtures.

## Modelling

### *Effect of Temperature*

Enzyme activity is generally measured as the amount of some specific substrate converted per unit time. This activity, as observed by activity measurements, is a combination of a true concentration of the enzyme, multiplied by its specific reaction rate constant. The specific rate constant increases with increasing temperature according to Arrhenius law (eq. 5). At still higher temperatures the enzyme starts to denature (second reaction eq. 1), thereby effectively decreasing the amount or concentration of the active enzyme configuration. Eventually, the enzyme loses its activity entirely. The rate constant of that part of the enzyme, that is still active, however, continues to increase with temperature. This principle has been used, verified and calibrated for a number of enzymes in a variety of products (Ponne et al 1996, Tijskens et al. 1997-1999).

The fundamental assumptions in the development of a model on enzyme activity as a function of temperature, at both activating as well as denaturing (Whitaker 1994 p. 303) temperatures, are:



From this (simplified) mechanism a set of differential equations can be derived, based on the general rules of chemical kinetics:

$$\begin{aligned} \frac{\partial En}{\partial t} &= -k_d En \\ \frac{\partial S}{\partial t} &= -k_s En S \\ \frac{\partial P}{\partial t} &= k_s En S \\ \frac{\partial En_{na}}{\partial t} &= k_d En \end{aligned} \quad (\text{eq. 2})$$

The general solution for this set of differential equations at constant conditions of temperature is for the active enzyme configuration En:

$$En = En_0 e^{-k_d t} \quad (\text{eq. 3a})$$

The apparent activity Act (as measured in experiments) is, as already mentioned, represented by the product of enzyme concentration (En from eq. 3a) and the specific activity ( $k_s$ ) (Godfrey & West 1996, p 491):

$$Act = k_s En = k_s En_0 e^{-k_d t} \quad (\text{eq. 3b})$$

In this equation  $k_s$  and  $En_0$  only appear in combination with each other. It is therefore impossible to estimate both variables at the same time. Both parameters are therefore combined in a new parameter called  $Act_0$ . This results in the final equation:

$$Act = Act_0 e^{-k_d t} \quad (\text{eq. 4})$$

In all these equations, the rate constants  $k_s$  and  $k_d$ , and hence also Act and  $Act_0$ , depend on

temperature according to Arrhenius law:

$$k = k_{\text{ref}} e^{\left( \frac{E_a}{R} \left( \frac{1}{T_{\text{ref}}} - \frac{1}{T} \right) \right)} \quad (\text{eq. 5})$$

With the combined equations 4 and 5, the activity of an enzyme can be described and predicted, as already reported (Ponne et al 1996, Tijssens et al. 1997-1999).

### **Effect of pH**

The effect of acid  $\text{H}^+$  ions or basic  $\text{OH}^-$  ions on the activity of an enzyme is probably caused by a change in stereo configuration at or in the neighbourhood of the active sites (Fersht 1984 chapter 5, Whitaker 1994 chapter 10). As in almost all protonation reactions, these reactions will occur very fast. The different configurations are instantaneously in equilibrium. The protonated and hydroxylated enzymes are assumed to be completely inactive or at least less active. This can be represented by the following mechanisms:



where  $K_{\text{EH}}$  and  $K_{\text{EOH}}$  are the equilibrium constants of the reactions. The water dissociation is defined as usual as:

$$K_w = \text{H}^+ \text{OH}^- \cong 10^{-14} \quad \text{and} \quad \text{OH}^- = \frac{K_w}{\text{H}^+} \quad (\text{eq. 7})$$

The amount of  $\text{EnH}^+$  and  $\text{EnOH}^-$  can now be expressed in terms of actual amount of active enzyme and pH by:

$$\begin{aligned} \text{EnOH}^- &= \frac{K_w \cdot \text{En}}{K_{\text{EOH}} \cdot \text{H}^+} \\ \text{EnH}^+ &= \frac{\text{En} \cdot \text{H}^+}{K_{\text{EH}}} \end{aligned} \quad (\text{eq. 8})$$

The total amount of enzyme in any configuration has to remain constant:

$$\text{En}_{\text{tot}} = \text{EnH}^+ + \text{EnOH}^- + \text{En} \quad (\text{eq. 9})$$

Combining the equations 8 and 9, and solving for En, an expression is obtained for the active enzyme at any  $\text{H}^+$  concentration (or pH):

$$\text{En} = \frac{\text{En}_{\text{tot}}}{1 + \frac{\text{H}^+}{K_{\text{EH}}} + \frac{K_w}{K_{\text{EOH}}} \cdot \frac{1}{\text{H}^+}} \quad (\text{eq. 10a})$$

Again this model for the amount of available active enzyme configuration, can be converted into an apparent activity (Act) as for the temperature model. This results in:

$$\text{Act} = \frac{k_s \text{En}_{\text{tot}}}{1 + \frac{\text{H}^+}{K_{\text{EH}}} + \frac{K_w}{K_{\text{EOH}}} \cdot \frac{1}{\text{H}^+}} \quad (\text{eq. 10b})$$

Again, in this equation  $k_s$  and  $\text{En}_{\text{tot}}$  only appear in combination with each other. It is therefore impossible to estimate both variables at the same time. Both parameters are therefore combined in a new parameter called  $\text{Act}_0$ . This results in the final equation:

$$\text{Act} = \frac{\text{Act}_0}{1 + \frac{\text{H}^+}{K_{\text{EH}}} + \frac{K_{\text{w}}}{K_{\text{EOH}} \cdot \text{H}^+}} \quad (\text{eq. 11})$$

Equation 11 describes how the activity of the total pool of enzyme changes with  $\text{H}^+$  concentration, over the complete range from pH 0 up to pH 14. Whitaker (1994) and Fersht (1984) deduced this equation in a somewhat different form. All equilibrium constants in this equation ( $K_{\text{EH}}$ ,  $K_{\text{EOH}}$ ,  $K_{\text{w}}$ ) may depend on temperature, according to Arrhenius law (eq. 5). Although it is generally considered to be and used as constant, the water dissociation constant  $K_{\text{w}}$  does indeed depend on temperature with an activation energy of about 74 kJ/mol (estimated based on data from Chang, 1981). As a consequence, the neutral pH changes from 7 at 24 °C to about 6.7 at 40 °C. How this affects the pH dependent activity of enzyme is not yet known.

### Combining Effects of Temperature and pH

In the temperature model (eq. 4 combined with eq. 5), no effect of pH was assumed, and the active configuration only decays by heat treatments into an inactive configuration. In the pH model (eq. 11) no denaturation of any of the three configurations was assumed and all effects of pH were implicitly assumed to be reversible. To combine the dependencies on pH and temperature into one model, we have to realise that denaturation could occur for each of the pH dependent configurations. The denaturation by heat of the active site of enzymes is supposed to consist of an irreversible change of the configuration at that active site. This change in configuration is most probably related to the folding and/or unfolding of the protein backbone, affecting directly the accessibility of the active site. Given this argument, and assuming that the effect of  $\text{H}^+$  and  $\text{OH}^-$  on the stereo-configuration is small, all three pH dependent configurations would denature as a function of temperature with the same rate. The consequence of this assumption is that  $\text{En}_{\text{tot}}$  in eq. 11 will decrease according to eq. 3. This decrease in enzyme concentration is reflected in the measured activity at that temperature time combination.

So, we can simply combine both equations (eq. 3 and 11) to obtain a model description for the combined effects of temperature on pH:

$$\text{Act} = \frac{\text{Act}_{\text{tot},0} e^{-k_d t}}{1 + \frac{\text{H}^+}{K_{\text{EH}}} + \frac{K_{\text{w}}}{K_{\text{EOH}} \cdot \text{H}^+}} \quad (\text{eq. 12})$$

With this equation, combined with the law of Arrhenius (eq. 5), we can describe and eventually predict the behaviour of the activity of enzymes in general at all combinations of denaturation time, denaturation temperature and pH. The derivation of the equations has been conducted under clear and plausible assumptions, thereby ensuring that all parameters in the equation have a very distinct and defined meaning, completely within the normal paradigm of chemical kinetics. This set-up ensures that theory and practice can develop further, and all disadvantages of empirical models have been avoided.

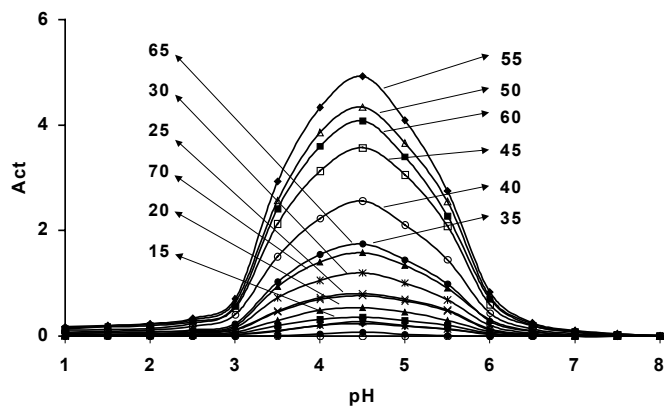


Figure 3-1 Measured raw data of the activity of phytase, produced by *E. coli*

## Results and Discussion

### **Classical Analysis of Activity of Phytase**

To get a feeling of the data, and of the behaviour of phytase activity one set of data was plotted as a function of pH for each temperature separate (see Figure 3-1). One can clearly see an increase in activity at pH rising up to about 4.5 pH. Below a pH of 2 and above 7 all activity is apparently vanished.

The sequence in temperature indicates a gradual increase in activity from 10 to 55 °C followed by a rapid decline of activity at higher temperatures.

Each of the data sets were first analysed in a classical way using non-linear regression (Genstat, Rothamsted) for each temperature separately, applying the derived formulation (eq. 10). The parameters  $Act_0$ ,  $K_{EH}$  and  $K_{EOH}$  were estimated explicitly without including effects of temperature as described by eq. 5.

The data were given a weight of the activity itself, to ensure the statistical system can put more weight to measurable quantities of activity and to avoid too large an effect of the many measured zero and almost zero activities. A value of 0.001 was however added, to avoid losing the zero measured activity completely. As a consequence of applying this weight to the data, however, the lower values of activity will be estimated less accurately.

As an example, the results of this classical analysis for phytase produced by *E. coli*, are shown in Table 3-2. The percentage variance accounted for is at each temperature well above 97 % and in most cases (with meaningful activity behaviour) above 98%.

The estimated values of the two equilibrium constants  $K_{EH}$  and  $K_{EOH}$  (see Table 3-2) appear to be constant at all temperatures. They are apparently completely independent of temperature. Except again for the temperature series with too little information: the series at the lowest temperature and the two highest temperatures contain relatively a large number of observations with zero activity, thereby inducing a statistical error in the estimates.

Since the  $K_{EH}$  and  $K_{EOH}$  parameters are truly equilibrium constants, their dependence on temperature should indeed be smaller than for rate constants. The dependence on temperature of equilibrium constants is governed by a difference in activation energy of the two constituting rate constants (see eq. 3). Assuming that the energy of activation of both the forward and the backward reaction is not too much different, the equilibrium constant is virtually independent of temperature.

Table 3-2 Result of classical analysis for phytase produce by *E. coli*

Temp	$Act_0$	$K_{EH}$	$K_{EOH}$	$R^2_{adj}$
10	0.328237	1.719636E-04	5.779097E+08	97.5
15	0.452460	2.517238E-04	4.186113E+08	98.4
20	0.696012	2.438420E-04	4.553575E+08	98.9
25	0.994116	2.878767E-04	3.540954E+08	98.2
30	1.476519	2.875450E-04	3.980221E+08	98.3
35	2.182364	2.702215E-04	4.277430E+08	98.4
40	3.165687	2.709816E-04	4.193693E+08	98.6
45	4.401291	2.762502E-04	3.825113E+08	98.5
50	5.358138	2.794840E-04	3.915411E+08	98.5
55	6.123231	2.725963E-04	4.279555E+08	98.6
60	5.089296	2.707086E-04	4.288763E+08	98.6
65	1.959778	2.787064E-04	3.994817E+08	98.6
70	0.945208	2.933519E-04	3.484279E+08	97.9
75	0.410177	1.181121E-04	7.774358E+08	97.0
80	1.381726	3.981685E-06	2.703618E+10	97.9

From the experimental set-up measuring the activity of phytase at the treatment temperature, it can be expected that the main effect of temperature is to be found in the value of  $Act_0$ , which represents, as already mentioned the apparent activity of the enzyme. This reflects the

increase in enzyme activity by temperature activation and the decrease in amount of enzyme present by heat denaturation caused by the rising temperatures, as described by the derived equation of Act (eq. 4). In Figure 3-2 the behaviour as a function of temperature, of the estimated values of  $Act_0$  for phytase produced by *E. coli*, is shown. The behaviour of the estimated values of  $Act_0$  closely resembles the general pattern observed in the measured activity at each of the temperature levels (see Figure 3-3).

The values for  $Act_0$  estimated for each temperature, excluding the two lowest and the two highest temperatures, were analysed with temperature as explaining variable, using eq. 4 and 5 in a non-linear regression analysis. In this way an estimate of the magnitude of the temperature dependence (activation energy) of the  $k_s$  part in  $Act_0$  and  $k_d$  was obtained.

### Combined Analysis of Phytase Activity

Non-linear regression analysis is an iterative procedure that relies very much on a good and reliable initial value for the parameters to be estimated, especially with complex equations and interactions. To obtain these reliable initial values, a sequence of non-linear regression analyses was performed, first using the values of  $K_{EH}$  and  $K_{EOH}$  as determined, estimating successively  $k_{s,ref}$  and  $E_s$  and subsequently  $k_{d,ref}$  and  $E_d$ . This sequence was repeated twice.

After this cycle of analyses, good and reliable values for all 6 parameters were obtained. In the final analysis, starting with the just obtained initial values, all six parameters were estimated simultaneously. In all these analyses, again a weight factor of activity was used. In Table 3-4 the results of the combined and final analysis for each of the data sets are shown.

The overall percentage variance accounted for ( $R^2_{adj}$ ) on all data per data set simultaneously ranges from 97.6% to 99.5%. The standard errors of estimate (s.e) are accordingly small. The measured and simulated data for phytase produced by *E. coli* is shown in Figure 3-6.

The scatter plot is shown in Figure 3-4. A deviation of the ideal line (45° angle) can be observed for low activities. This is of course caused by measuring difficulties at these ranges of low activity, and by the application of a weight, equal to the activity: deviations in the lower activity range are considered less important and more acceptable.

The residuals (see Figure 3-5) reflect roughly the same behaviour: although the deviations are rather small, the relative larger deviations occur at lower activities. Some indications exist in the residual plot that some trends in the data are still not fully explained. Apparently, there still is some effect of either pH or temperature left in the residuals. The data, however, are well described by the model (see  $R^2_{adj}$  in Table 3-4) and the remaining effects are considered not important enough for further analysis.

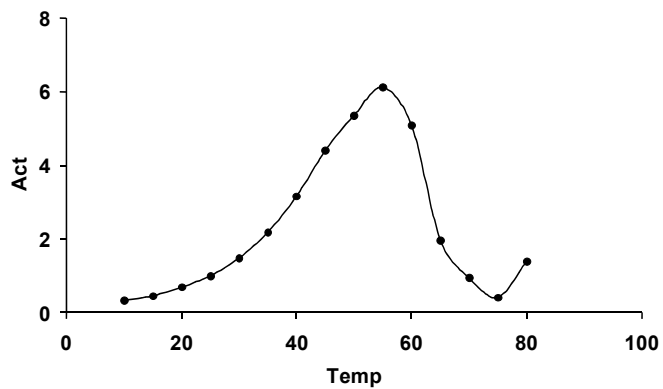


Figure 3-2 Behaviour of the estimated values of  $Act_0$  as a function of temperature for phytase produced by *E. coli*.

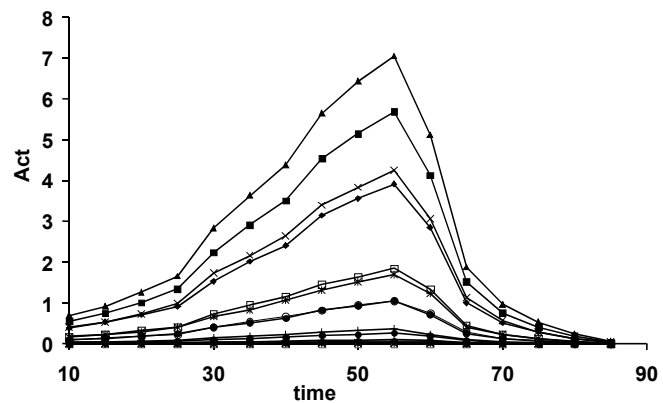


Figure 3-3 Activity of phytase as a function of temperature (Barley P2) for each of the pH levels (1 to 8)



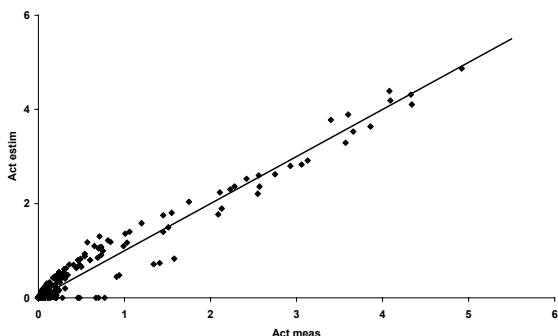


Figure 3-4 Scatter plot for the activity of phytase produced by *E. coli*

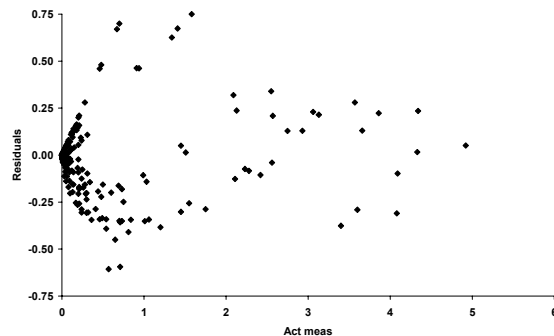


Figure 3-5 Residual plot for the activity of phytase produced by *E. coli*

### Optimal pH of enzyme activity

Based on the deduced equation of simple pH dependence of enzyme activity (eq. 11), the optimal pH of that enzyme can be easily determined. When we take the differential of eq. 11 with respect to  $H^+$ , put it equal to 0, solve the expression for  $H^+$ , and convert the expression to the  $-\log_{10}$  system as is usual for pH and equilibrium constants pK, we obtain:

$$pH_{opt} = \frac{pK_w + pK_{EH} - pK_{EOH}}{2} \quad (\text{eq. 13})$$

Up to now, two main types of phytases have been identified: acidic phytases with a pH optimum around pH 5.5 and alkaline phytases with an pH optimum around pH 8.0. All phytases used in this investigation belong to the acidic phytases. In Table 3-4 the calculated optimal pH for phytase activity of each of the sample origins is shown. The optimal pH ranges from 4.5 to 5.9 and they are in very good agreement

with the experimentally determined values (see Table 3-3). This is a rather surprising large range for the same type of enzyme from different origin. Even if all phytases used in this investigation belong to the same type of enzyme, there are significant differences between the phytases from micro-organisms and cereals. The microbial enzymes are more pH- and heat stable than their plant counterparts. In contrast to the cereal phytases, the microbial ones are rather specific enzymes and exhibit the highest turnover number with phytate. The cereal phytases exhibit a broad affinity for various phosphorylated compounds and the highest relative rates of hydrolysis were found with pyrophosphate and ATP.

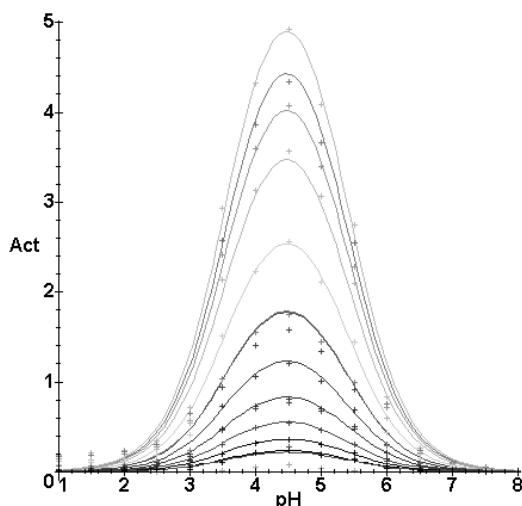


Figure 3-6 Measured (symbols) and simulated (lines) activity for phytase produced by *E. coli*

Table 3-3 Simulated and experimental Optimal pH of Phytase activity

Phytase type	pH <sub>opt</sub> Sim.	pH <sub>opt</sub> Exp.	Reference
<i>E. coli</i>	4.47	4.5	Greiner et al. 1993
<i>Klebsiella terrigena</i>	4.95	5.0	Greiner et al. 1997
Barley P1	4.97	5.0	Greiner et al. 2000
Oat	5.01	5.0	Greiner and Larsson Alminger 1999
Rye	5.63	6.0	Greiner et al. 1998
Barley P2	5.83	6.0	Greiner et al. 2000
Spelt D21	5.91	6.0	Konietzny et al. 1995

## Conclusions

The basic assumptions used in the development of this model seem to be valid. The model describes accurately the enzyme activity as a function of pH and temperature simultaneously over a very wide range in temperature and a wide range in pH. The normal rules of chemical kinetics and equilibria remain valid and can be further applied.

All parameters have a fundamental definition and meaning, and exhibit properties and values that can be expected for this type of parameters.

The activity of phytase of each of the studied origins complies fully with the model description. The experimental set-up, measuring the enzyme activity at treatment temperature and a large and meticulous matrix of experimental conditions, ensures a reliable analysis, covering the full dynamic space of activation and denaturation by heat and configuration conversion by pH.

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3: The effect of temperature and pH on the activity of enzymes: the case of phytases

Table 3-4 Analysis of Phytase activity as a function of pH and Temp

Estimate	$K_{EH}$	$K_{EOH}$	$Act_{tot,0}$	$E_s/R$	$k_{d,ref}$	$E_d/R$	$R^2_{adj}$	$N_{obs}$
Barley P1	2.143E-05	1.852E-09	22.350	7984.0	0.038770	11235.0	98.0	240
Barley P2	4.793E-06	2.200E-08	7.137	5165.0	0.001158	20260.0	97.6	272
<i>E. coli</i>	2.746E-04	2.433E-09	3.272	7255.0	0.002356	17283.0	98.9	240
<i>Klebsiella terrigena</i>	1.071E-04	8.400E-09	2.960	4653.8	0.000102	26722.0	98.4	255
Oat	2.302E-05	2.400E-09	40.690	12848.0	0.107300	9717.0	99.5	195
Rye	1.405E-05	2.500E-08	14.920	4907.0	0.027400	6652.0	98.2	272
Spelt D21	8.790E-06	5.900E-08	9.426	11070.0	0.038220	10474.0	97.6	727
s.e.	$K_{EH}$	$K_{EOH}$	$Act_{tot,0}$	$E_s/R$	$k_{d,ref}$	$E_d/R$		
Barley P1	1.250E-06	3.195E-08	1.990	433.0	0.005420	703.0		
Barley P2	2.550E-07	1.200E-09	0.191	173.0	0.000274	1002.0		
<i>E. coli</i>	8.850E-06	7.692E-08	0.042	193.0	0.000370	605.0		
<i>Klebsiella terrigena</i>	3.750E-06	2.900E-10	0.036	99.4	0.000027	1028.0		
Oat	na	na	Na	na	na	na		
Rye	5.520E-07	9.500E-10	1.050	263.0	0.004330	398.0		
Spelt D21	na	na	Na	na	na	na		
$T_{ref}$ °C	40							

## **Part 2**

### **ELUSIVE QUALITY:**

### **A PHILOSOPHY OF QUALITY**

## Introduction

In one way or another all research and technological efforts in agriculture are related to quality. Massive efforts have been conducted to produce quality, to maintain quality as good as possible, and in the long run, to improve the quality of our lives. It is, however, amazing that this ubiquitous and paramount quality is so ill defined, and differs from person to person, from situation to situation. In working with quality of agricultural produce, researchers are implicitly, and most often unknowingly involved in the psychology of man, and his ever-changing behaviour, his ever-changing desires and the ever-changing fulfilment of these desires. So, for conducting fundamental research on product behaviour and product properties with the ultimate goal of understanding product quality, it is mandatory to at least realise this dichotomy.

So, we have to grasp the meaning quality has for each individual consumer, how he translates his perception of the product and how he evaluates his perceptions. This whole process will eventually result in an acceptance of the product by an individual consumer for an individual purpose (fulfilling expectations MacFie et al. 1995, Meiselman et al. 1996). Furthermore, the behaviour and decisions of individual consumers with respect to acceptability and actual acceptance, is strongly affected by the economic boundaries and market situation in which that particular consumer operates.

For research on quality, acceptance, product behaviour and consumer behavioural patterns targeted at practical applications in production, trade and processing of food products, this individual perception and evaluation of quality has to be extended to include group behaviour, both for the product as for the consumer. That is the realm of consumer research and market research and is well out of the scope of this work. Nevertheless, to provide some information to consumer research and market research, and to provide some guidelines for product research, food production, trade and processing, it would be very advantageous to uncouple these three interacting fields associated with what is normally called quality.

Also for modelling product behaviour, with respect to quality for users and consumers, it is essential to have at least a fundamental notion what quality really is, and which product properties determine the quality assigned to a product by the consumer. In other words, what is allowed and what is to be avoided when modelling product behaviour in terms of quality and acceptability?

In this part, a more philosophical view on quality is worked out, developed with the sole purpose of determining how and what to model when describing food quality as objectively as possible. Meanwhile, it turned out to be a satisfactory theory for quality in general (see e.g. Tijssens and Vollebregt 2003). The central and crucial aspect of the viewpoint is the decomposition of evaluation and appreciation into a more (although not completely) objective assessment of quality, called the assigned quality, which is valid for the majority of consumers and users. The more subjective appreciation in terms of acceptability is postponed to the market and consumer part of the acceptance chain.

In Chapter 4 the concepts of the philosophical view are highlighted, based on a clear distinction between product properties and quality attributes, perception and evaluation, appreciation and acceptance. The main conclusion of this study with respect to quality is that what is called quality in day-to-day life, is more often than not acceptance. In scientific reports, the word quality should be avoided altogether to reduce misunderstanding amongst highly different individuals.

In chapter 5 an overview is given of existing consistent viewpoints on quality as perceived from different perspectives. Also rules and mechanisms are sought and reviewed to combine multiple quality attributes into one single "indicator" of quality.

In the last chapter of this part, the principles and viewpoints mentioned above are brought to the area of acceptance and acceptability, which are closer to the consumer behaviour research than to the product area. Both items have a higher impact in the field of marketing research and logistics.

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**4**

**Concepts for Modelling Quality of Perishable Products**

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Published in *Trends in Food Science & Technology*, 1996, **7**, 165-171

## Abstract

It is generally accepted that the quality of perishables products depends on three factors, the product, the user, and the market situation. It is therefore difficult to define what quality is and how to control it. Decomposing the effects of these factors on quality leads to a distinction between the assigned quality and the acceptability of a product. Assigned quality is the quality notion a consumer has of a product and results from evaluating that product with respect to his criteria. Acceptability defines whether the consumer in that particular situation is willing to buy that product. It is the result of relating the assigned quality to other products and to extrinsic factors such as the price. Product and consumer research focus on assigned quality, while market research focuses on product acceptability. Changes in assigned quality can be simulated with quality change models, which consist of separate models for the quality assignment, for the product behaviour, and for the product environment.

## Introduction

From the moment of harvest, agricultural products have a limited life because of loss of quality during the period between harvest and consumption, even when the products are distributed at optimal conditions. This loss of quality may be large if products are not treated optimally.

Quality is becoming an increasingly important marketing factor both for producers and consumers. So, during distribution of agricultural products, management of quality is very important. Because of this growing importance, definitions for quality have been developed within various areas of research. These approaches to quality are reviewed below.

Based on these approaches, a conceptual model of quality is presented, which incorporates an explicit decomposition of the factors that affect quality: the user of the product, the product itself, and the environmental conditions to which the product is subjected.

Next, a quality change model is defined as a composition of separate submodels for each of the quality-determining factors. Finally, the advantages of this approach are described for analysis and modelling of quality and quality change of agricultural products.

## Approaches to quality

Quality is a very elusive concept that depends on many factors. In the first place, quality depends on the product itself. Quality also depends on the preferences of the user. The preferences may arise from the intended use of the product (e.g. ripe tomatoes for soup, hard tomatoes for salads), and from social-psychological factors such as the user's attitude towards the product. For example, one person may be status-conscious and prefer plum tomatoes from Italy, another may be environmentally aware and prefer organically grown tomatoes. A third aspect that may affect quality, is the market situation: the quality of a product depends on its price (a higher price is often taken to indicate higher quality) and on the availability of other, competing, products (a product of moderate quality will be assigned a higher quality when surrounded by products of poor quality, than when surrounded by products of high quality).

Several approaches to defining quality reflect these different aspects (Garvin 1984, Steenkamp 1989). These approaches stem from the areas of philosophy, production management, economics, and consumer research.

### **Philosophy**

The *metaphysical* or *transcendent* approach views quality as an unanalysable property that a user can only learn to recognise through experience. Because people acquire different experiences, their quality evaluations are bound to be different. This approach serves more as background knowledge to the concept of quality than as a practical method to handle quality.

### **Production management**

The *production management* approach is concerned with maintaining quality during

production and uses technical specifications to objectify product quality: a product that conforms to the technical specifications has a high quality. Production starts with product design, includes manufacture and distribution, and extends to maintenance and after sales services. For each stage in the production process, specific quality criteria are used to monitor and control that production stage (Juran, Gryna and Bingham 1974).

Although this approach was developed for non-perishable products, the concepts relating to the design and the production stages can be applied to agricultural products as well. Breeding new cultivars with properties such as a better resistance to certain diseases or a better taste, can be regarded as improving the quality of design. Production of agricultural products consists of a growth phase and a distribution phase. Examples of maintaining quality during production are control of the growth conditions, and the use of packaging throughout a complete distribution chain.

### **Economics**

Economic theories of producer and consumer behaviour in markets containing products with differing quality use a *product-based* definition of quality. Quality in this case is the composite of product characteristics.

Theories about producer behaviour describe how producers use quality to maximise their profits by differentiating their products from competing products. Differentiation can be achieved (1) by changing the value of a quantitative characteristic, e.g. by increasing the amount of vitamins in a food product, or (2) by making the product more appealing to a specific group of consumers, e.g. harvesting fruits at different time points, so that consumers can choose between ripe and unripe fruits, or (3) by introducing a new quality attribute that eventually may make existing quality grades obsolete, e.g. organically growing instead of using fertilisers.

Economic theories about consumer behaviour assume that consumers try to buy those goods that have the highest quality. Lancaster (1971) defines quality as 'those objectively measurable, technical properties of goods that are relevant to consumer choice'. Different consumers may perceive these properties differently. In Lancaster's model the differences in perception are captured in individual preference functions.

Many economic theories assume that consumers are completely informed about the price and quality of the products available on the market. This assumption is, however, unrealistic. Consumers are most of the time imperfectly informed and, therefore, use various strategies to evaluate the quality of available alternatives. Depending on how the quality of a product is determined, three types of strategies can be distinguished. The first is to *search* for a product with the highest quality by inspection of available products prior to purchase. An example is comparing available wines by using descriptions of the bouquets. The second strategy to evaluate the quality of product alternatives is by *experience*: by trying different alternatives and selecting that alternative that provides the largest benefit. An example is to buy and taste different wines, until a wine is found with the most favourable bouquet. Some attributes may not be evaluated by actual experience with the product. For these so-called *credence* attributes consumers must rely on information from external sources. An example of a credence attribute is the percentage alcohol in the wine.

### **Consumer research**

The *user-based* or *perceived-quality* approach puts the user in the central position. In this approach quality is considered to be subjective: it depends on the perceptions, needs and goals of the individual user. The term '*perceived* quality' emphasises this. Also the term 'fitness for use' (Juran, Gryna and Bingham 1974) expresses that quality depends on the user.

Several definitions for perceived quality have been proposed (Steenkamp, 1989, Kramer Twigg 1983). Kramer and Twigg (1983) define quality as 'the composite of those characteristics that differentiate individual units of a product, and have significance in determining the degree of acceptability of that unit by the buyer'. The difference between user-based and product-based definitions of quality is that the product characteristics need not be measurable any more. A user-based definition may refer to product characteristics

that in reality do not exist, but that the user believes to be important.

Steenkamp (1989) distinguishes between quality cues and quality attributes. Quality cues are those product related characteristics that are ascertained prior to consumption. Quality cues are similar to the search attributes of Lancaster (1971), and can be intrinsic or extrinsic (Olson and Jacoby 1972). Intrinsic quality cues are part of the product, and cannot be changed without also changing the nature of the product. Examples are firmness and colour. Extrinsic quality cues are related to, but not part of, the product. Examples are brand name and price. Quality attributes are only observable during or after consumption. Two types of quality attributes are distinguished: *experience attributes* and *credence attributes*, with the same meaning as in Lancaster's economic model of consumer behaviour.

### A conceptual model of quality and quality change

In the approaches described in the previous section, the quality of a product depends on both intrinsic and extrinsic product properties. The intrinsic product properties define the state of the product, which is set off against quality criteria imposed by a producer (product management approach) or a user (consumer research approach). Extrinsic product properties, such as the price and the quality-price ratios of the product and of other products, are used as additional information in the decision whether or not to purchase the product.

The distinction in the use of intrinsic and extrinsic product properties can be extended into a distinction between the *assigned quality* of a product and the *acceptability* of a product. This is illustrated in Figure 4-1.

*Assigned quality* is the result of an evaluation

of a product only with respect to the intrinsic product properties. Assigned quality specifies the suitability of the individual product to the needs and goals of a user, without referring to extrinsic properties of the product, or to other products. The needs and goals of the user are reflected in the criteria, which the user imposes on the intrinsic product properties, when assigning quality to the product. As an example, a different criterion will be applied to the firmness of a tomato according to whether it will be used in a soup or in a salad: for soup only ripe tomatoes are suitable, while for salads only hard tomatoes are. Therefore, ripe tomatoes have a high assigned quality when the user wants to make tomato soup, but the same tomatoes will have a low assigned quality, if the user wants to use them in a salad.

The combination of the assigned quality, the extrinsic product properties, and the market situation yields the *acceptability* of a product: an assessment of the product in relation to its price and to other products. Independent of the assigned quality of the product, the acceptability will decrease if other products available are assigned a better or worse quality. The acceptability of a product corresponds to what Garvin (1984) calls 'affordable excellence', quality in terms of costs and price. This *value-based* approach to quality is often difficult to apply, as it combines a measure of excellence (quality) with a measure of value (price).

From this perspective, the approaches reviewed in Section 2 describe strategies used by consumers in deciding whether to accept a product (the economic theories about consumer behaviour and the consumer research approach), and strategies used by producers to increase the acceptability of their products (the economic theories about producer behaviour and the production management approach). The concept of perceived quality used in consumer research differs from assigned quality, in that the perceived quality depends also on extrinsic product properties and on the market situation, while the assigned quality

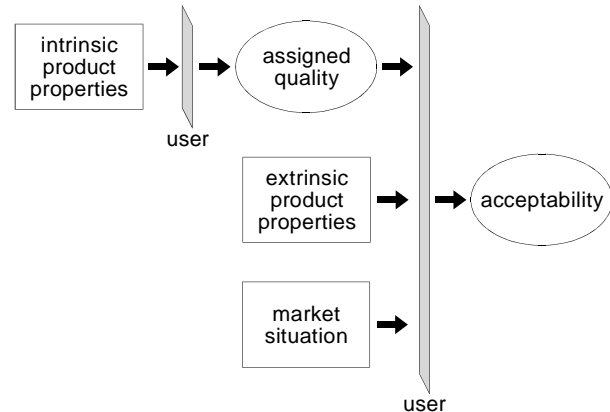


Figure 4-1 A user evaluates intrinsic product properties to assign quality to a product. By also taking into account extrinsic product properties and the market situation, the user determines the acceptability of the product.

depends solely on intrinsic product properties.

Assigned quality may change because of changes in intrinsic product properties, or because of changes in the criteria imposed on these product properties. This distinction between changes in product behaviour and in quality criteria can also be found in the model on keeping quality, described by Tijskens (Tijskens 1995, Tijskens and Polderdijk 1996). Keeping quality is defined as “the time, a product remains acceptable under whatever circumstances and using whatever acceptance limits”. Like assigned quality and perceived quality, is keeping quality a combination of the product behaviour and of the (possibly changing) quality criteria. Keeping quality, however, differs from assigned quality and perceived quality, because the latter represent assessments of a product at a certain point in time, while keeping quality represents the period that all quality attributes of the product comply with the quality criteria.

Changes in the intrinsic properties of agricultural products may be caused by conditions in the environment, to which the products are subjected during post-harvest storage and distribution. The environmental conditions themselves can be affected by the product, particularly for packaged products, e.g. respiring fruits releasing gases. In this case, a strong bi-directional interaction exists between the product and its environment. For non-packaged products, only a unidirectional interaction is important, as in that case, the influence of the product on its environment is negligible.

From this line of reasoning, the changes in the assigned quality of agricultural products can be decomposed into three quality-determining factors: the assignment of quality to a product by the user, the changes in the intrinsic product properties, and the interaction between the product and its environment.

### **Quality assignment by the user**

Users select certain quality attributes and impose criteria on these attributes to assign quality to a product (see Section 2). The quality attributes selected by a user, and the criteria imposed on these attributes form the *quality notion* of the user with respect to a certain product. Although each user may in principle have a different notion of quality, groups of users can be identified that use the same quality attributes in their evaluations, and impose more or less equal criteria on these quality attributes. We call such groups *homogeneous* with respect to assignment of quality to a certain product. In modelling quality change, we define quality assignment with respect to such homogeneous groups of users, for example an expert panel, rather than with respect to an individual user.

Assignment of quality to a product is a process that transforms in several steps the many intrinsic properties of a product into one (subjective) uni-dimensional measure of quality. To arrive at an assignment of quality, a user perceives and evaluates a number of intrinsic product properties, and then carries out an appreciation of these evaluations, see Figure 4-2. In the rest of this section these three steps are explained in more detail.

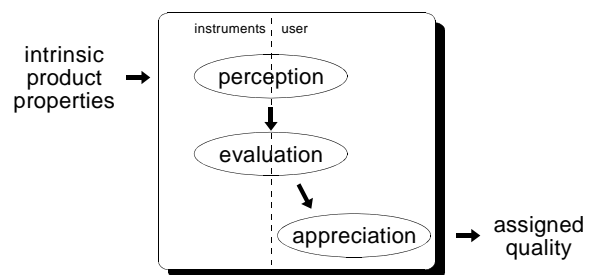


Figure 4-2 Steps in quality assignment to a product by a user. For the perception and evaluation steps both instruments and human senses may be used, the appreciation is entirely done in the mind of the user.

### **Perception**

The first step is the perception of the intrinsic product properties. Properties of perishable products can be perceived either by instruments (e.g. firmness measurement by a penetrometer or an Instron, colour measurement by a colour meter) or by human senses (e.g. pressing a tomato between your fingers). Some properties, such as the amount of vitamin C, can only be assessed by instruments. These are the so-called hidden attributes (Kramer and Twigg 1983). Other properties can (up to now) best be assessed by human senses (e.g. flavour). Through perception, the properties are converted into quality attributes. A quality attribute can be based on several product properties. A good example is colour,

which in most cases is the perception of the concentrations of several colour components inside the product.

Sensory perception is complex. Even mealiness, which is an apparently straightforward quality attribute of apples, does not show a one-to-one relationship with the amount of cell juice, but depends also on how the apple tissue fractures when bitten. Mealiness is enhanced by fracture along cell walls thereby preventing the perception of the juice and the sugars present in the intact cell. A user would therefore experience a mealy apple as dry, although the apple may contain almost the same amount of juice as a crispy apple.

#### **Evaluation**

In the second step, the perceived quality attributes are evaluated to determine their intensities or values. Evaluation can also be conducted both by instruments and by human senses. As perception and evaluation are strongly connected, perception and evaluation of a quality attribute are usually performed with the same 'equipment', i.e. instruments or human senses.

The relation between a stimulus intensity and the corresponding sensation experienced by the human senses is not a simple linear one. It generally flattens at high intensities due to saturation of the human senses, while intensities below a certain threshold intensity of the user, will not be perceived at all.

Another characteristic of using human senses instead of instruments to evaluate quality attributes, is a possible shift in perceived intensity after evaluating several products. A quality attribute may be evaluated differently after products with low intensities for that attribute than after a batch of products with high intensities.

#### **Appreciation**

Once the quality aspects are perceived and evaluated, they can be converted into appreciations. In many cases, the relation between evaluation and appreciation of a quality aspect shows a strong optimum: after the first increase in liking with increasing intensity, the curve flattens in a region of no preference, followed by a more or less steep decline in liking with increasing intensity. A very weak salt solution or distilled water is not very agreeable. Neither is a very strong salt solution.

Finally, the appreciations of the individual quality attributes are combined into a uni-dimensional quality measure. In this step, relative weights are assigned to the individual quality attributes and to combinations of quality attributes. These weights reflect the influence of socio-psychological factors, such as personal preferences, fashion, tradition, and status symbols, on the assignment of quality to a product. The socio-psychological factors determine the attributes to be used, and an order of importance of these attributes.

#### **Describing a product state**

During the quality assignment, users evaluate and appreciate quality attributes that are perceptions of product properties.

Just as in consumer research quality cues are described as being intrinsic or extrinsic (see Section *Consumer Research*), so we can categorise product properties as being extrinsic or intrinsic. For example, intrinsic properties of mushrooms are: species, growing origin and condition, amount of water in the mushrooms, firmness, colour, while the price, the appearance of the package, and the shop where the mushrooms are bought, are extrinsic.

A categorisation in product properties can also be made depending on whether or not the properties change during the normal lifetime of the product. Properties that change during the lifetime of the product are called *variable product properties*. Properties that are constant are called *fixed product properties*. Of the mushroom properties given above, growing origin and species are fixed product properties. The amount of water, firmness and colour are variable product properties.

A third distinction is whether the value of the product property can effectively be controlled or manipulated. This is more an issue for the operational and strategic planning of distribution of (agricultural) products than for modelling or understanding their post-harvest behaviour. Operational planning concerns the performance of activities during distribution, and

therefore, only concerns those variable product properties that can effectively be manipulated. Strategic planning, however, involves the (re)design of distribution chains, in which case also properties that are fixed during the lifetime of the products, such as harvest time, may be manipulated.

For the purpose of quality change modelling, only intrinsic product properties are relevant. Of these, the values of the variable product properties at any point in the lifetime of the product determine the *product state*. The change in the product is a series of such product states at successive time points. With each product state is associated an assigned quality, determined by the user through the perception, evaluation, and appreciation of the product, as described in Section *Quality Assignment by the Consumer*. Hence, the quality change of the product can easily be determined, given the time series of product states.

#### ***Behaviour of a product***

During the normal lifetime of a product, the variable intrinsic product properties change due to processes occurring in the product. Examples of such processes are the (further) ripening of fruits, and the opening of flower buds. Many processes are complex systems of chemical reactions (respiration, colour development), while other processes have a physical nature (water uptake through the flower stem). Still other processes have both chemical and physical aspects, such as the complex process affecting the firmness of a product. Firmness may be described as a combination of turgor pressure, which is a physical quantity, and of concentrations of various chemical compounds like pectines, which are affected by chemical reactions.

Each process causes changes in one or more variable product properties. The action of a process may be affected by external factors, such as environment temperature, and by other product properties, both fixed and variable. Thus, a variable product property affected by one process may influence the action of another process in the product, acting on another variable product property. These patterns of interactions between processes result in the observed complex physiological behaviour of agricultural products.

During the lifetime of a product, processes may be activated or inactivated. For example, during the distribution of vegetables, packed in Modified Air packages, the respiration, and as a consequence the deterioration, gradually decreases by the low oxygen, but increases quite suddenly again when the package is opened. Denaturation of enzymes during blanching is another example of a process getting inactivated. The enzymatic process cannot be activated any more as the enzyme denaturation is irreversible.

Apart from such discrete events as opening the MAP package, a process may also become activated or inactivated as a consequence of a continuous change in the product. For example, many fruits in the pre-mature stage will ripen slowly until they reach the climacteric stage. When reaching the climacteric stage (a hyper active state in many fruits just before ripening), the rates of ripening processes will increase, so that the effects of these processes become important.

#### ***The environment of a product***

As stated above, many processes in agricultural products are affected by conditions in the environment immediately surrounding the product. The product environment can be represented by *external factors*, of which the most important are temperature, relative humidity, and the concentrations of oxygen, carbon dioxide and ethylene.

The environment of a product may be affected by processes occurring in the product: respiration affects the oxygen and carbon dioxide concentrations, evaporation increases the relative humidity, and heat production changes the temperature in the environment. The effect of the product on the environment will become relevant when the product is contained in a relatively small closed space, for example in (MAP) packaging. Then, the environment may become unfavourable for the quality of the product, e.g. evaporation of the product wrapped in foils may cause the relative humidity inside the foil to rise, causing fungal infections (Van der Sman et al. 1996). On the other hand, in Modified Atmosphere

Packaging, the package material is designed to exploit the processes in the product to bring about an environment that is favourable for minimising quality change (Kader, Zagory and Kerbel 1989).

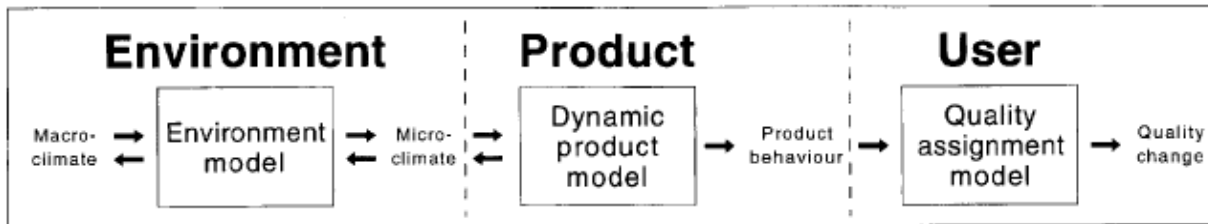


Figure 4-3 A quality change model is a composition of three separate submodels for the entities affecting the changes in assigned quality: the quality assignment by the user, the physiological behaviour of the product in a certain environment, and the changes in this environment.

### Definition of a quality change model

To model the changes in quality, assigned to a product subjected to certain environmental conditions, three separate models are needed for the three quality-determining entities, quality assignment, product behaviour, and product environment. These three models and their interactions are depicted in Figure 4-3. The models will be described from right to left.

#### **The quality assignment model**

The *Quality Assignment Model* (QAM) describes how a defined (homogeneous) group of users assigns quality to a certain product. The QAM specifies the product properties that are relevant for that particular group of users. For each relevant product property the QAM specifies the relation between the product property and its appreciation by the group of users. Furthermore, the QAM contains a *quality function*, comparable to the preference function in the model of Lancaster (1971) that combines the appreciations of the individual product properties into one uni-dimensional measure of quality.

Wilkinson and Polderdijk (Wilkinson and Polderdijk 1995) developed a Quality Assignment Model for the assignment of quality to tulip bulbs by various user groups in the Dutch tulip bulb chain. In this model, the quality function was of the form of a summation over all the quality attributes, their square and all interactions, each sum weighted by a weight factor. In this case the weight factors are based on the results of questionnaires sent to members of the links in the distribution chain. The first summation (over the individual quality attributes) represents the quality attributes with linearly increasing or decreasing appreciations, such as bulb damage and bulb disease (less damage and less disease always give a higher quality). The second summation (over the square of individual quality attributes) represents the quality attributes with an optimum appreciation. The last summation (over the product of all individual quality attributes) represents appreciations of combinations of quality attributes. For bulb quality, such an interaction exists between bulb damage and bulb disease: if bulbs are damaged less weight is given to the presence or absence of disease. This reflects the knowledge of the users that a damaged bulb is more susceptible to disease whether or not any disease is visible.

As a QAM only describes how one specific group of users assigns quality to a certain product, several quality assignment models have to be used to describe quality assignment by different groups of users. As the product behaviour does not depend on the quality assignment, these different quality assignment models can be connected to one dynamic product model. In the case of tulip bulbs, two quality assignment models are defined, depending on the intended usage of the bulbs. One model is for bulbs destined for 'dry sales' directly to the consumer, while the other is for bulbs destined for the production of cut flowers ('forcing'). In both models quality assignment is described primarily as a linear function of bulb damage and bulb disease. The quality assignment model for forcing puts more weight to disease than the one for dry sales.



### ***The dynamic product model***

The changes in the product properties represent the behaviour of the product in its environment. The *Dynamic Product Model* (DPM) describes how environmental conditions affect product behaviour. This model consists of several submodels, each describing one process occurring in the product or an aspect of a process. An example of how complex physiological behaviour can be decomposed into the constituting subprocesses, which are then described in separate submodels, can be found in the chilling injury model of Tijskens, Otma and van Kooten (1994). The detrimental effect of radicals (process 1), generated in and outside the product (process 2), is prevented by a radical scavenging system (process 3), which deteriorates at lower temperatures (process 4). The reaction rates of these four subprocesses all depend on temperature according to Arrhenius' law (process 5). Each subprocess describes a small but well-defined part of the behaviour of intrinsic product properties. The initial conditions and boundaries together with the generic model formulation explain and describe the various forms that chilling injury as a process and as a property behaviour can show.

### ***The environment model***

An *environment model* describes the changes in the environment of the product. If the product is packaged, the environment model describes the changes in the conditions inside the package (the micro-climate), as they are affected by physical processes such as diffusion of gases through the foil or the package, and by processes in the packaged product such as respiration, evaporation, and heat production.

In the case of non-packaged products, an environment model will generally reduce to a series of environmental conditions at successive points in time.

## **Conclusions**

The quality of a perishable product depends on characteristics of the product itself, on criteria imposed by the user of the product on these characteristics, and on other alternative products. These three factors lead to the complex behaviour of quality observed during post-harvest distribution of perishable products. The concepts of assigned quality and of acceptability of a product have been introduced, in which the effects of these three quality-determining factors are explicitly separated.

Assigned quality is an evaluation of the state of a product at some point in time. The product state is only determined by intrinsic product properties, which are in turn influenced by the environment. The product state is evaluated against quality criteria, which reflect the needs and goals of the user for that product. The assigned quality therefore depends on three factors: the user of the product, the intrinsic properties of the product itself, and the interaction between the product and its environment.

The acceptability of a product is an evaluation of the assigned quality in the context of extrinsic properties of the product such as its price, and in relation to other available products. Product acceptability includes a trade-off between price, availability and quality, whereas for the assigned quality itself the price and the availability of other products are not relevant.

The changes in quality assigned to a product can be formalised and analysed in a quality change model. Such models consist of three submodels: one describing the quality assignment by the user, one describing the physiological behaviour of the product, and one describing the change in the environment of the product.

Using separate submodels has several advantages. Firstly, the separation of product behaviour and quality assignment allows a description of the phenomena occurring in a product, independent of the user's attitude, and enables reuse of the same product model for different user groups. The separation also allows a clear description of the quality notions of users.

Separating the changes in the environment from the product behaviour has a similar advantage. In most literature, however, the processes occurring in the package and the

behaviour of the packed products are combined into one model. Such models thus directly link the product behaviour to the conditions outside the package. Separate modelling of the environment and of the product leads to a clearer conceptual description, and enables reuse of both the package model and the product model.

Secondly, different analysis and modelling techniques may be used for the three entities. The environment model describes physical processes, while the product model describes complex biochemical processes. Quality assignment has a psychological nature, for which black-box modelling may be more appropriate.

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**5**

**Modelling Food Quality**

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Published as Chapter 17 in Tijskens L.M.M., Hertog M.L.A.T.M., Nicolaï B.M. (eds.) (2001).  
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## Introduction

Quality is becoming increasingly important as a determinant of food choice. As disposable income in the developed world increases, the influence of price on food choice decreases and factors such as healthfulness, convenience and quality become more important. Quality is particularly important in determining repeat purchases of food products (Steenkamp and van Trijp, 1996). Competing on quality rather than on price often has advantages from the point of view of companies. It creates customer loyalty, raises barriers to competition and reduces price elasticity (Steenkamp, 1990). However a quality based strategy can only succeed if the company knows what the customer understands by a good quality product and if the company can translate these demands into (technical) product specifications. In this chapter an approach to food quality modelling is described which will allow companies to *understand* the relationship between perceived quality and product characteristics, to *predict and control* quality during production and distribution, and to *optimise* quality during product development.

However first it is necessary to define what is meant by quality. Already since Roman times it is well known that quality and taste are something personal and specific to every individual human being: 'de gustibus et coloribus non est disputandum', or 'do not discuss colour and taste'. Although the information, contained within the product upon which we all judge the quality of that product, is the same for everybody, the interpretation and the appreciation can be very different for different people. So a workable definition of quality has to cover both aspects: the general information in the product and the specific effect the product properties exert on different people.

A frequently used definition is that given by Juran (1974), "fitness for use".

Kramer and Twigg (1970) provide as definition of quality: "the composite of those characteristics that differentiate individual units of a product, and have significance in determining the degree of acceptability of that unit by the buyer".

Steenkamp (1990) gives a more elaborate definition: "Perceived quality is an idiosyncratic value judgement with respect to the fitness for consumption which is based upon the conscious and/or unconscious processing of quality cues in relation to relevant quality attributes within the context of significant personal and situational variables."

Implicit in all three definitions is that quality is the result of the interaction between the person and the product. It will depend not only on the characteristics of the product (colour, sugar content...) but also of the person (age, culture...) and of the context (meal, dish...). There is no one objective measure of quality and this is emphasised by authors who use terms like 'perceived quality' (Steenkamp, 1990) or 'assigned quality' (Sloof et al., 1996).

Another implication of these definitions of quality is that modelling of food quality will require the collaboration of disciplines both in the social sciences (for example, consumer scientists, market researchers and economists) and in the natural sciences (for example, biochemists and physiologists).

## Key principles and methods

### ***Process of quality assignment***

In order to model food quality it is important to understand how quality assignment takes place. The description that follows draws largely, though not exclusively, on the work of Steenkamp (1989, 1990) and Sloof et al. (1996, 1999).

Prior to consuming a food product (for example, when purchasing it in the shop) an individual forms an opinion as to the quality of the product, termed the *expected quality*. The individual bases the expected quality on *quality cues*. A quality cue is an attribute of the food product, which can be perceived before purchase and consumption and which is believed to be indicative of its quality. The individual believes the cue to be highly correlated with product quality, a belief that can be based on personal experience or on information from acquaintances or the media. Examples of cues are country of origin ('the best olives come from Italy'), price ('good wines tend to be more expensive') or colour ('dark red meat has a

better quality than pale meat’).

Only when the product has been consumed can the individual form a final opinion about the quality, the *experienced quality*. This unidimensional measure of perceived quality is a function of the *quality attributes*. Quality attributes are all those product attributes, which are relevant for determining the quality. Which product attributes are quality attributes thus depends on the priorities of the individual who assigns the experienced quality. The quality attributes can be divided into experience attributes which are determined before and during usage (flavour, ease of preparation...) and credence attributes which are based on beliefs (nutritional value, production methods, food safety...). The beliefs in turn can be based on information on the packaging, on information obtained from the media or from personal contacts.

Furthermore a distinction can be made for both quality attributes and quality cues between intrinsic attributes/cues and extrinsic attributes/cues. An intrinsic attribute or cue is one which cannot be changed without changing the product itself. Examples are the taste, the vitamin content, the size. An extrinsic attribute or cue is one, which is not part of the physical product, for example the price, the packaging, the brand or the supermarket where the product is purchased. Extrinsic attributes and cues are largely the domain of the marketing department. While not denying their importance for quality assignment, most quality modelling has concentrated on the intrinsic attributes and cues.

Some authors distinguish a third integration step in which an overall quality is assigned based on both the expected and the experienced quality (Poulsen et al., 1996). This allows for situations where disconfirmation of expectations leads to an overall quality assignment, which is different from the experienced quality. For example, the appearance of the packaging may lead to the expectation of a certain taste and therefore quality. If this is confirmed by experienced quality, then satisfaction ensues. However if the taste is less favourable than expected, then this will lead to negative disconfirmation, which may lead to the product being rejected in a situation where, if expectations had been lower, it would have been accepted (Andani and MacFie, 2000). It follows from this that producers need to pay attention not only to quality attributes but also to quality cues and their relationship with quality attributes. The interplay of attributes, cues and quality is illustrated in Figure 5-1.

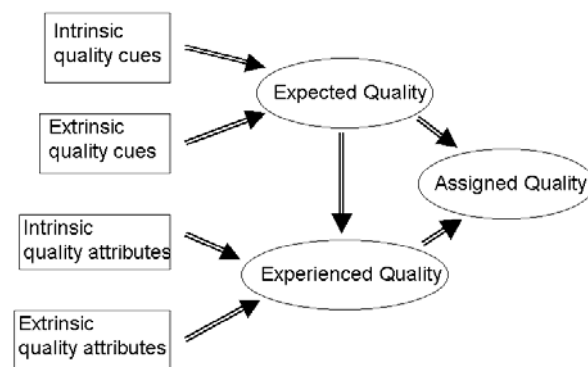


Figure 5-1 Quality assignment based on intrinsic and extrinsic quality cues and quality attributes.

A concept, which is closely related to quality, is that of acceptability. When the individual decides on the acceptability of a product, he compares the (expected) quality to some criterion, termed the “quality limit” (Tijskens, 2000). If the quality exceeds the quality limit, he accepts the product, otherwise he rejects the product. This quality limit is dependent on the personal preferences and situation of the individual. Acceptability is involved in the ‘keeping quality’ (Tijskens and Polderdijk, 1996) which is so important for perishable products. For perishable products such as fruit and vegetables, the quality attributes change (usually deteriorate) over time. Keeping quality is a measure of the time it takes before the assigned quality falls below the quality limit at any condition during storage and transport. Shelf life is the keeping quality under specified storage conditions (Tijskens, 2000). It follows from the above that keeping quality is no objective measure but depends on the priorities and preferences of the individual. Certainly producers should take account of differences between countries and between market segments.

For quality modelling it is necessary to consider how intrinsic quality attributes and cues are related to the product properties, defined as the physico-chemical characteristics of the

product. A single attribute can be a function of several product properties. For example, the quality attribute 'perceived sweetness' can be a function not only of the amount of sugars but also of acids (Lawless and Heymann, 1998). Several distinct steps can be identified in arriving at the quality attribute or cue (Sloof, 1999, Sloof, Tijssens and Wilkinson, 1996, Tijssens, Sloof, Wilkinson, 1994). Firstly the product properties form stimuli, which are perceived by the human senses. For example, the taste receptors on the tongue are triggered by the acid and sugar components in the food product. Secondly these perceptions are integrated and evaluated to form an evaluation of the intensity of the quality attribute or cue. In our example, the information from the taste receptors is combined to form an evaluation of how sweet the product is. In the final appreciation step a hedonic judgement takes place. In the terms of the example, the product is judged to be not sweet enough, just right or too sweet. These steps are summarised in Figure 5-2.

### Quality Assignment Model

#### Introduction to quality model

If the aim of food quality modelling is summarised as the modelling of the effects of choice of cultivar or recipe, temperature and other external factors during storage and processing on perceived quality then it is clear from the above that a number of steps are involved. As is illustrated in Figure 5-3, the relationship between external factors and product properties, the relationship between product properties and quality attributes/cues and the relationship between quality attributes/cues and perceived quality all need to be described. This leads naturally to the decomposition of the task of food quality modelling into a number of sub-tasks (see also Chapter 2). This is best achieved by decomposing the food quality model into two main sub-tasks (Sloof, 1999):

- A Quality Assignment Model (QAM) describing the relationship between quality attributes/cues and perceived quality. This model should also consider the influence of situation and characteristics of the individual (age, culture...) on quality assignment.
- A Dynamic Product Model (DPM) describing the effect of external factors such as choice of cultivar or recipe, temperature during storage and processing on product properties and therefore quality attributes/cues.

Approaches to develop quantitative models will now be discussed for both of the sub-tasks.

## Product

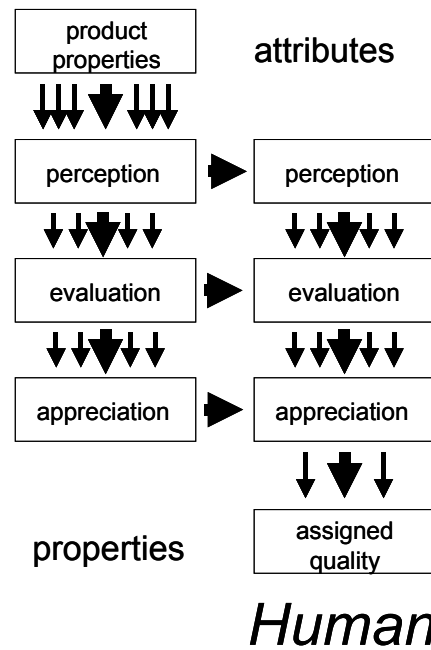


Figure 5-2 Steps in assigning quality to a product by a human

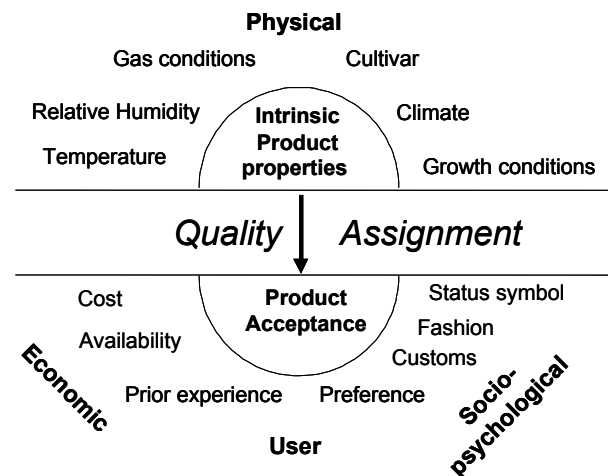


Figure 5-3 Quality assignment as affected by product properties, economic and socio-psychological circumstances

*Product centred approach*

Molnár (1995) has developed a mathematical model to describe quality assignment. He distinguishes five categories of attributes, namely

- sensory properties
- chemical composition and physical properties
- microbiological contaminants,
- toxicological contaminants
- packaging, labelling, shelflife.

The relative importance of each category will vary according to the food product. For example, for functional foods the category “sensory properties” may be judged relatively unimportant. Within each category, relevant attributes are identified based on the knowledge of experts. Each attribute  $x_i$  is normalised to give  $z_i$  with  $1 \geq z_i \geq 0$ .  $z_i$  receives the value 1 when  $x_i$  is at its optimum value and the value 0 when  $x_i$  is at its worst value or becomes unacceptable. In fact,  $x_i$  represents the evaluation of the attribute and  $z_i$  the appreciation of the attribute. To arrive at total quality, each normalised attribute is assigned a weight, and the attribute categories are also assigned weights, giving the mathematical function

$$Q = \sum_{j=1}^{n_{\text{cat}}} W_j \sum_{i=1}^{n_j} w_i z_i \quad \text{eq. 1}$$

where

$Q$  is total quality,

$W_j, j = 1 \dots n_{\text{cat}}$  are the weights for the attribute categories

$w_i, i = 1 \dots n_j$  are the weights for the individual attributes of category  $j$ ,

$z_i, i = 1 \dots n_j$  are the normalised attributes.

Molnár defines “primary critical” attributes as attributes whose zero value indicates that the product is unfit for human consumption and that the total quality is therefore zero. Examples of primary critical attributes are sensory off-flavour attributes and food safety related attributes.

Again Molnár suggests that experts are used to determine the weights for the quality function. The fact that Molnár does not use consumers to gain information about quality attributes and the quality function is a weak point of his approach. It is the consumer who will finally assign a quality to the product and it is unlikely that the expert can fully interpret the priorities of the consumer. It is known that experts tend to consider quality as compliance with technical specifications and to lay excessive emphasis on the absence of defects compared to consumers (Tijssens, Sloof, Wilkinson, 1994, Lawless and Heynmann, 1998).

*Consumer centred approach*

A number of researchers operating in the field of marketing and consumer research have developed and quantified models for quality assignment which do place the consumer centrally (Acebrón and Dopico, 2000, Steenkamp and van Trijp, 1996, Poulsen et al., 1996). The models are built around the description of the process of quality assignment given above and thus include quality cues and expected quality as well as attributes and experienced and overall quality. The quality assignment model is quantified for a particular product by means of consumer research. Typically consumers are asked to assess uncooked, packaged products and assign scores to a pre-determined list of quality cues and to expected quality. Then they are presented with (or prepare themselves) the cooked product. Again they assess the product and assign scores to quality attributes and experienced quality. Sometimes they may be asked to assign a final overall quality score. The quality scores are related to the attribute and cue scores using a statistical technique such as linear regression, Partial Least Squares regression (Steenkamp and van Trijp, 1996) or LISREL (Poulsen et al., 1996). This gives for each step in the quality assignment process one quantitative formula, which describes quality assignment for all consumers. It is interesting to see that although the consumer research approach to quality assignment modelling places the consumer at the centre, no allowance is made for the subjective nature of assigned quality and for differences

between consumers in their quality model.

It is not always clear in these models whether consumers are scoring the appreciation or the evaluation of the attributes and cues. In Poulsen et al. (1996) consumers are asked to rate the sensory attributes of cookies on a nine-point scale ranging from “much less than ideal” through “ideal” to “much more than ideal”. This is clearly measuring appreciation. However Acebrón and Dopico (2000) ask consumers to rate cooked beef on a four-point scale from very tough to very tender. This is an evaluation and it seems to be implicitly assumed that appreciation is positively and linearly related with tenderness.

There are two problems with using consumers to provide evaluations rather than appreciations. Firstly it is an unnatural task for consumers. People are naturally able to say how much they like a particular attribute but intensive training (as a product expert, as a member of a sensory analytical panel) is required before they are reproducibly able to score how much there is present of a particular attribute. Secondly the relationship between the evaluated amount and the appreciation is often non-linear (as reflected in the scale used by Poulsen et al., 1996). The relationship between the appreciation of individual attributes and total assigned quality is probably monotone and it is plausible to assume that it is approximately linear. The modelling of linear relationships is much simpler and much less demanding in terms of the amount of data required.

It can also be questioned whether asking consumers to score both quality cues/attributes and expected/experienced quality at the same moment on the same product will give reliable results. It can lead to socially desirable answers, with high fat or high sugar content products receiving artificially low quality scores. Also because pre-determined lists are used based on expert knowledge, it may bring the attention of the consumer to an attribute or cue which in a real-life situation would have played no part in his quality assignment. An alternative approach would be to allow the consumer to score the attributes or cues at a different moment (but for an identical product) to the moment at which the quality is scored.

### *Statistical techniques*

Two techniques, which allow the influence of individual cues and attributes on, assigned quality to be deduced indirectly are conjoint analysis and preference mapping. Both techniques were not originally intended for developing quality models but can be used for that purpose.

Conjoint analysis was developed to determine price elasticity. In conjoint analysis the respondent is presented with written descriptions of a set of products and is asked to rank or score them in order of preference (Johnson, 1974). The products are described according to a limited number of attributes with each attribute taking one of a fixed number of values according to a factorial experimental design. The respondent does not rank or assign scores to the individual attributes but rather to the (described) product as a whole. By relating the rankings or scores for the products to the attributes varied in the experimental design (for example, using regression) it is possible to estimate the effect of each attribute on preference. Conjoint analysis has been used to build quality functions for ham (Steenkamp, 1987) and flower bulbs (Wilkinson et al., 1994, Wilkinson and Polderdijk, 1996).

The aim of preference mapping is to model the relationship between sensory profiles of products and consumer preferences. Consumers are presented with a set of physical products. They judge each product and assign it a score according to their preference. At the same time a trained analytical sensory panel evaluates the values of a set of sensory attributes for the same set of products to provide a sensory profile for each product.

Consumer preferences are related to sensory profiles using linear or nonlinear regression models, which allow an estimation of which sensory profiles are preferred. A strong point of preference mapping is that the preferences of each individual consumer are modelled separately. This leads naturally to a recognition of the subjective nature of preference and to segmentation, clustering of groups of consumers with similar preferences. While preference mapping is designed to elicit information about the influence of sensory attributes, the technique could in theory be extended to include the influence of other categories of attributes.



### *Summary Quality Assignment Models*

To summarise, a number of approaches have been described for developing Quality Assignment models. The consumer research approach is the one, which keeps closest to the description of the process of quality assignment. However, it could be improved by incorporating features from other approaches. These include the definition of primary critical attributes and the decomposition of attributes into categories with differing importance, as proposed by Molnár, the indirect measurement of attribute and cue appreciation as used in conjoint analysis and preference mapping, and the modelling of quality assignment at the individual consumer level, analogous to the preference mapping approach.

### ***Dynamic Product Model***

The Dynamic Product Model aims to describe the relationship between external factors and product quality attributes or cues. It is possible to decompose the model still further to separately model the relationship between external factors and product properties, as well as the relationship between the product properties and the perceived quality attributes or cues. For example, one model could describe the relationship between storage or processing variables and the sugar content of French fries, and a second model could describe the relationship between the sugar content and the colour. In fact this is rarely done, with most authors directly modelling the quality attribute or cue (though see Hertog et al. 1997).

The most common approach is to build a model for a single attribute or cue. Often these are primary critical attributes, according to the definition of Molnár, such as spoilage. Models are frequently developed to predict keeping quality as determined by spoilage. As discussed earlier, keeping quality is in fact the time until quality falls below an acceptable level, the quality limit. An example is the model described in Hertog et al. (1999) which describes spoilage of strawberries due to Botrytis infection as a function of temperature and gas conditions. In Zwietering (1993) and Zwietering and Rombouts (1994) kinetic models are discussed which describe bacterial growth and which can be used to predict keeping quality. In addition to models for primary critical attributes, models have also been developed for other attributes. The choice of attribute is often influenced by the priorities of the producer rather than the consumer. Marcelis and Gijzen (1998) developed production (growth) models which predict the fresh weight of cucumber fruits. They consider this to be an important quality attribute because it determines market price. This reflects an outlook on quality from the point of view of the producer and distributor rather than the consumer. Such an outlook also leads to an emphasis on quality cues, which determine expected quality at the point of sale rather than experienced quality attributes. As an example, Vankerschaver et al. (1996) developed a model for the visual quality of cut endive but not for its taste.

A major application of Dynamic Product Models is in simulation studies. They can show the effect of changes in storage and processing conditions on key quality attributes and can thus be used to optimise logistic chains or food processing. For example, Hertog et al. (1999) used simulations to gain insight into the effect of logistic chains and packaging on spoilage of strawberries. For true optimisation, Dynamic Product Models need to be combined with Quality Assignment models. This is not yet done. However in simulations, some direct information about assigned quality is obtained, as it can be seen when the quality of a product is no longer acceptable.

### ***Integration of Quality Assignment Model and Dynamic Product Model***

While most modelling takes place within either the Quality Assignment sub-task or the Dynamic Product sub-task, the Quality Function Deployment and the Quality Guidance frameworks do overstep this boundary (Viaene and Januszewska, 1999, Steenkamp and van Trijp, 1996, Poulsen et al., 1996, Bech, 2000). Both frameworks comprise a set of models, which relate consumer quality preferences via quality cues and attributes to technical product specifications. However they are more than just a collection of models as they embody a philosophy in which product development is guided by the voice of the consumer. They are tools to improve the collaboration between marketing and R&D departments in the process of new product development.

The Quality Function Deployment framework defines a series of research steps to carry out

this goal. Firstly potential market segments are defined and consumer demands are identified. Secondly consumer preferences are translated into technical and sensory product specifications. These relationships are summarised visually in a set of pictorial matrices which collectively form the so-called House of Quality (Bech, 2000). This can be used to predict consumer perceptions of existing own and competing products and new concepts. The predictions can be validated using the results of consumer research. An example of the use of Quality Function Deployment for the chocolate industry can be found in Viaene and Januszewska (1999).

The Quality Guidance framework extends the consumer research approach to modelling Quality assignment (see above) with a step relating perceived quality attributes and cues to product properties. These relationships are inherently multivariate. One quality attribute may be a function of several product properties. For example, the meat quality attribute 'appearance' may be influenced by colour, amount of fat and moisture. Equally one product property may affect several quality attributes. Pâté coarseness may contribute positively to the attribute 'taste' but negatively to the attribute 'leanness' (Steenkamp and van Trijp, 1996). To describe these multivariate relationships, Partial Least Squares Regression (Steenkamp and van Trijp, 1996) or LISREL (Poulsen et al., 1996) can be used.

### **Areas of application**

The two main areas of application for food quality models are in quality control and product development.

#### ***Quality control***

In quality control an already existing product forms the basis. The aim of the producer is to ensure a constant and high quality at all times. This is achieved by monitoring the product during production and distribution and taking the necessary actions to maintain its quality. Because quality control does not have the intention of altering the existing product, quality is primarily interpreted as meaning conformance to technical specifications and avoidance of defects. This does not necessarily mean that the subjective nature of quality is ignored. Thus in quality control of rice it is recognised that quality indices for rice intended for the Japanese market will differ to that for the American market due to differences in taste preferences and use of rice in meals - Japanese prefer sticky rice served plain, Americans prefer non-sticky rice served with a sauce (Barton et al., 1998). However it can mean that quality control tends to emphasise the avoidance of negative attributes and cues (for example external defects) rather than maximising positive attributes and cues (for example, a good taste). Also technical specifications have a lack of flexibility which means that they do not always reflect the sometimes rapid changes in consumer preferences.

Food quality modelling can be implemented for three different purposes in quality control.

- *Prediction of key quality attributes using instrumental measurements.* Often at-line, in-line or on-line instrumental measurements are routinely carried out at production and distribution facilities. These measurements of product properties are then related to key quality attributes/cues using calibration models. Examples are instrumental measurements of cucumber colour, of pea mealiness, of the fat content of French fries and of rice protein content. There is an increasing use of non-invasive spectroscopic and other multi-parameter instruments, indicative of an increasing recognition that a given quality attribute or cue is generally a function of several product properties. The application of instrumental measurements would benefit from Quality Assignment models. These could be used to direct attention to those quality aspects, which are truly found to be important by consumers rather than aspects which producers think consumers find important. Also the use of quality Assignment models can help avoid excessive emphasis on quality cues rather than quality attributes. Moreover they could be used to determine which values of quality attributes or cues are appreciated, and by which groups (segments) of consumers. This would ultimately enable production tailored to the quality demands of specific segments in the market.

- *Prediction of effect of raw material properties on final product quality.* For producers of processed food there is a need to determine and control the effect of raw material characteristics on final product quality. With this information they can reward suppliers by paying more for 'high quality' raw materials. This is done for example in the French fries industry where the amount paid out to growers for potatoes is partly determined by the fry colour. Food producers can choose to vary their production process to compensate for variations in raw material characteristics and ensure a constant output quality. For some products (for example juice, port and blended whisky), producers can use these prediction models to optimally combine different batches of raw materials in order to obtain a constant and high quality. In most current practical applications, prediction models relating raw materials to final product quality are empirical, based on observed correlations under a given processing condition. Such prediction models gain in usefulness when they are based on Dynamic Product Models, which describe how processing conditions change the raw product to obtain a certain set of quality attributes (see Van Dijk and Tijssens, 2000).
- *Calculation of quality indices.* A quality index can be seen as the practical application of a Quality Assignment model. It provides a well-understood measure of the quality of a product which is recognised by all members of the production and trade, and allows products to be compared in an objective manner. An example for this kind of practical application of simplified Quality Assignment models is the Streif index for apple maturity at harvest. Developed in the early seventies (Streif 1976), the index is defined as the ratio between the firmness of apples and the Brix refraction times the starch stage. It has obtained an increasing application to determine the harvest date for apples, optimal for subsequent storage and eating quality. It is nowadays so widely used that complete conferences (De Jager et al. 1996) are devoted to its application, improvement and its calibration against sensory and expert data.

### **Product development**

In new product development both Quality Assignment models and Dynamic Product Models can be applied in the search for a product with the desired quality. The process of product development starts with the consumer - 'what does he want?', moves to the product - 'how do I make it?' and then back to the consumer - 'does he like what I made?'. Several iterations may be necessary. In the process, food quality modelling can be involved at all three stages.

- In the first stage Quality Assignment models can be applied to determine the quality preferences of consumers. The Quality Assignment models can show whether there is segmentation in quality perception, which attributes (and at which levels) determine quality perception and whether available products are currently meeting quality demands.
- In the second stage R&D is presented with a set of desired quality attributes and cues. At this stage Dynamic Product Models can be applied to determine what modifications in processing conditions or ingredients are required to achieve the desired attribute profile.
- In the third stage traditionally a consumer acceptance test is carried out on one or two products. However it is possible to use the Quality Assignment models developed in the first stage to predict the assigned quality for a greater range of potential products as a first screening.

The use of food quality modelling for new product development as described here is largely implemented in the Quality Function Deployment and Quality Guidance frameworks discussed in the previous section. However these frameworks give limited input for product design and optimisation (stage 2) as they stop at the translation of quality demands into product properties. These frameworks do not describe how these product properties should be modified. To achieve this, fundamental knowledge has to be generated and incorporated (see Chapter 2 and 3).

## Pros and Cons and Future trends

An advantage of food quality modelling is that it makes explicit the subjective nature of assigned quality, implying that the same product can have different perceived qualities for different people in different situations. As yet this aspect of quality modelling is not well incorporated in the methodology with most Quality Assignment models providing one mathematical function for all consumers, or for only one defined subgroup. The future will see more attention to the question of segmentation, in which quality assignment models are built for defined groups of consumers with similar quality demands and defined categories of situations. This will enable companies to tailor their products to the quality demands of defined groups of users.

Variations not only between consumers but also between individual product units within a batch - the so-called biological variance - will also receive more attention in the future (Tijssens et al. 2000). Regarding the Quality Assignment Model, the perceived quality of a batch obviously depends on the perceived quality of the individual units, but the nature of this relationship is not so obvious. Is it a simple average, or do poor quality units have extra weight? More research is needed on this perception question. Regarding the Dynamic Product Model, there are some interesting developments in modelling batch behaviour with a limited number of unit-dependent parameters (Nicolai et al. 1995, Nicolai and van Impe 1996, Tijssens and Wilkinson 1996, Tijssens 2000, see also chapter 6).

Quality Assignment models are inherently very broad, covering in their fullest form intrinsic and extrinsic attributes as well as cues. This is both an advantage and a potential problem. The advantage lies in the healthy counterbalance it offers to the tendency in the past to reduce quality to one or two easily measured aspects. Thus producers and distributors in the fruit and vegetable sector have in the past tended to concentrate on a limited number of mostly external attributes which could easily be measured instrumentally or by product experts (for example, colour and firmness but not taste). The broad range of the Quality Assignment models on the other hand presents big challenges for the consumer research methodology necessary to quantify the models. The mechanisms underlying the perception and appreciation of experience attributes such as taste are very different from those for credence attributes such as calcium content or extrinsic cues such as brand name. Sensory perception and appreciation is largely determined by the physical morphology, a result of genetic makeup and age. Belief in the importance of calcium is a cognitive attitude and much more susceptible to influence.

The extensive Quality Assignment models contrast too with the Dynamic Product Models. While the Quality Assignment models are highly multivariate, relating many cues and attributes to several scores of quality, and usually assume simple linear relationships, the Dynamic Product Models are predominantly univariate, but are increasingly treated in a multi-response approach (Chapter 3). They model single quality attributes using nonlinear models describing kinetic or other chemical or physical processes. Perhaps the biggest challenge for the future is the combination of these two different styles of modelling to produce workable models which can predict quality given processing and other conditions, or alternatively which can deduce optimal processing conditions given quality demands.

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**6**

**Acceptability**

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Published as Chapter 7 in Shewfelt R.L., Brückner B. (ed.) (2001). *Fruit and vegetable quality: an integrated view*. p. 125-143. Technomic Press, USA  
and lecture in COPERNICUS/CIPA final workshop *Shelf-life prediction for safety and quality of foods*. P. 193-202, Nov 1997, Wageningen, NL.

## Introduction

Quality of products, and most certainly quality of perishable products is very elusive and difficult to define. In almost any language, up to the Romans, the saying is known not to discuss colour and taste. This in fact means that every individual is entitled to judge the quality, and hence the acceptability, of any product according to his own standards, however unclear or ill defined. And that is a good and fundamental right in any democratic state.

However, production and distribution of perishable products tailored to each individual preference, can never be achieved in large-scale operations. So, the food industry has to develop standards of quality and of acceptability, that cover the preferences of large groups of individuals. Studying these groups, and establishing the preferences of these groups, is the task of consumer research and marketing research. Establishing general patterns in quality behaviour and their description belongs to product research. Translating the established preferences and the quality behaviour of perishable products simultaneously into a workable philosophy on consumers' view of quality belongs to nobody in particular but to everybody in general. In this chapter a number of examples will be provided and discussed that indicate the power of problem decomposition and process-oriented modelling in areas related not only to product properties, but also to consumer behaviour and preferences.

## Decomposition of quality

Many studies have been undertaken to catch the elusive quality into a coherent theory, applicable in many situations. The objectives range from consumer research (Meulenberg & van Trijp 1996, Meulenberg & Broens 1996, Wierenga 1980) risk research (Frewer et al. 1993/94) market research (Steenkamp 1987, 1989), product behaviour (Kramer & Twigg 1970) and quality control (Juran 1974). Tijskens et al. (1994b) combined all these aspects into one theory on product quality, product acceptance and acceptability. This theory was further extended by Sloof et al. (1996). They used the principle of problem decomposition to arrive at a flexible but workable system, set up primarily for the purpose of modelling quality and acceptability behaviour of perishable products. The main difference with previously developed systems is that the quality of a product is decomposed in assigned quality and acceptability as separate and distinct issues. The user/consumer assigns a quality to the product based on the intrinsic properties present in the product. Consequently this assigned quality is more or less independent of applied criteria. This assigned quality is in fact the acceptance of a product (see next paragraph) without reference to any criterion. The sequence of steps taking place in the assignment of quality is schematically represented in Figure 6-1. The consumer, with his personal preference, thrive for status and awareness of costs (among others), then judges the acceptability of a product based on this assigned quality with respect to a given

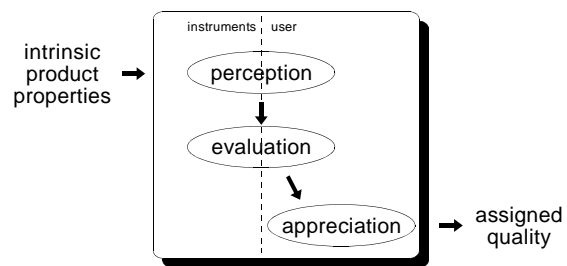


Figure 6-1 Quality is assigned to a product. The intrinsic properties are perceived and evaluated and an appreciation is determined.

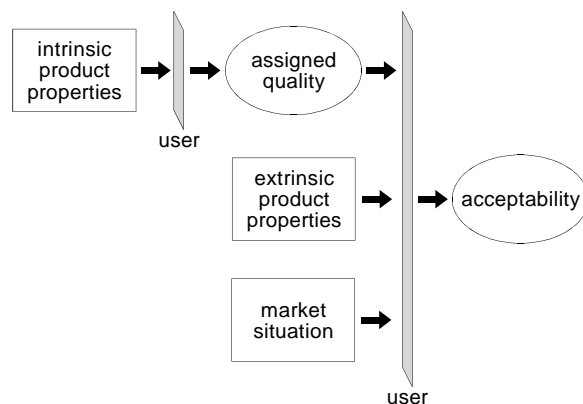


Figure 6-2 A user evaluates intrinsic properties to assign quality to a product. Taking extrinsic properties, his personal preferences and the market situation into account, acceptability is determined.



situation like price and availability (see Figure 6-2 and Figure 6-3).

## Acceptance and Acceptability

A subtle but distinct difference exists in the linguistic meaning of the words acceptance and acceptability. According to G. Hobson (personal communication) *acceptability suggests that some criterion is applied to differentiate those products that could be sold from those that could not, whereas acceptance does not imply any aspect involving a range in quality*. In other words, acceptance describes a general state of a product, whereas acceptability describes whether a particular product is accepted by a consumer in his particular circumstances of, e.g. status, price, personal preferences etc. as can be taken from the graphical representation (Figure 6-2 and Figure 6-3).

## Decomposition of acceptability

So, when we are talking about acceptability, some criterion has to be set to judge the general acceptance or assigned quality of a product. As such, acceptability covers both product aspects (product research) and consumer aspects (consumer research and market research) at the same time. To be properly applicable in practice, both types of aspects should be and can be studied separately without (too much) interference of one another.

Throughout this chapter this criterion is called **quality limit**. It stands for the criterion a consumer applies to the quality aspect involved determining the acceptability of that product.

### Consumer applied criteria

The criteria a particular consumer applies to the assigned quality in determining the product's acceptability are very frequently adapted to the situation of the individual. To obtain a more general view on acceptability criteria, the consumer research has to aim at particular groups of consumers, which roughly apply the same criteria in the same situation. Based on these groups of similar consumers the market strategies of companies selling commodities are in fact defined. Advertisement tries to affect the criteria applied by that specific group of consumers. Price policies try to lower the consumer's financial threshold. Hence, both efforts affect the acceptability of the product in a different way. All these actions, however, do alter neither the intrinsic properties nor the assigned quality. They have consequently, no effect on the description of product behaviour during growth (production), storage and processing. This signifies that the same dynamic product model can be used over and over again for the description and prediction of the intrinsic product quality at all conditions in the integrated view of fruit and vegetable quality.

### Assigned quality

So, the quality of a product and its dynamic behaviour during storage, transport and distribution can be based on the behaviour of all the intrinsic properties of a product that have a bearing in the quality perception of the consumer or a specific group of consumers. So, if we know which properties are used to arrive at an assigned quality (determination) and how they are used (relative importance), quality behaviour can be and may be modelled by modelling the appropriate properties. This information is known as the quality function (Tijskens et al. 1994b, Sloof et al. 1996). Many models have been developed, consciously or unconsciously based on this philosophy (Tijskens & Evelo 1994c, Tijskens et al. 1994a,

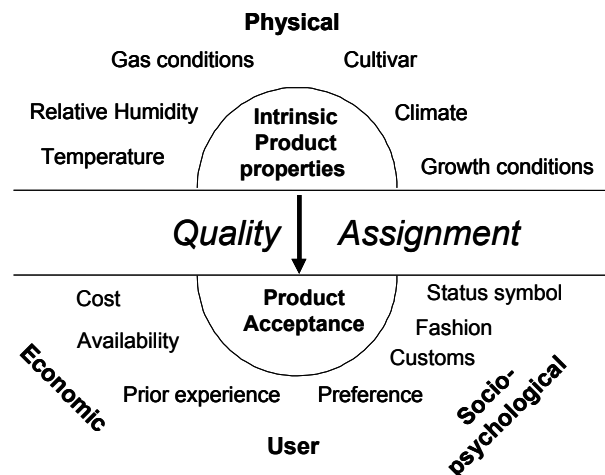


Figure 6-3 Relations between product properties, assigned quality and consumer's acceptance or appreciation.

1996a, 1996b, Verlinden 1996).

## Examples of Modelling Acceptability

When describing or modelling acceptability of (perishable) products, one has to realise that consumers can assign quality to each product item separately, e.g. the taste of one tomato. In acceptability, however, the quality aspect is combined with the economic aspect. Consumers may buy one individual tomato, commercial applications inherently cover complete batches of products. As such, within the aspect of acceptability a second aspect comes into play: How many individual items in a batch may be unacceptable before the batch as a whole becomes unacceptable? This aspect introduces a system of population dynamics in the description of batch behaviour.

### **Acceptability of potted plants**

The behaviour of acceptability of batches of products has been modelled for potted plants during storage in darkness (Tijskens et al. 1996b). In the assessments of the potted plants, the applied quality criteria and the quality limits were predefined and related among others to number of leaves and flower without defects. The limit of acceptability has therefore a fixed value and will consequently not be expressed explicitly in the equations used. As long as the selected quality criteria are applied consistently throughout the experiments, neither the type nor the value of the criteria for acceptability should be important for the development of the model. Acceptability is subject to local and regional preferences, hence, to ensure generic application, the model has to be independent of acceptability criteria. If the criteria of acceptability do not change during the assessment period, the decrease in acceptability is completely determined by the loss of those intrinsic product properties that contribute to the perceived quality.

The plants were given a standardised pretreatment, stored in darkness at six constant temperatures. Up to 21 days, quality was evaluated six times. The experimental conditions of time and temperature were chosen to simulate the conditions encountered during container transport to medium and distant markets.

#### *Time effects*

Loss of perceived quality over time is undoubtedly the result of a cascade of biochemical reactions. Such cascades can often be approximated by the sigmoid logistic curve. A logistic function is therefore likely to be a good model. The formulation for the logistic curve has been slightly adapted to enable the introduction of the effect of temperature in a way suitable for the dynamic approach:

$$N = \frac{N_{\max}}{1 + \left( \frac{N_{\max}}{N_0} - 1 \right) e^{kt}} \quad \text{eq. 1}$$

where  $N_{\max}$  = the number of plants in the batch,  $k$  is the rate of decrease in acceptability,  $t$  = the time in days,  $N$  = the number of acceptable plants,  $N_0$  = the initial number of acceptable plants.  $((N_{\max}/N_0)-1)$  constitutes a correction for the biological age of the plants at the start of the experiment (Thai et al. 1990, Tijskens & Evelo 1994c) and is of course independent of the temperature applied in the subsequent treatment.

#### *Temperature effects*

The rate  $k$  in equation 1 represents some combination of all biochemical reaction rates in a cascade of reactions. The rate  $k$  will therefore depend on temperature. The effect of temperature on the behaviour of potted plants can be twofold, with both high and low temperatures leading to a faster reduction in acceptability (see Figure 6-4 and Figure 6-5). This suggests two processes, one which is particularly active at high temperatures and results in, for example, excessive water loss, and one which is particularly active at low temperatures and results in, for example membrane deterioration and chilling injury (Tijskens et al. 1994a). The acceptability will decrease with an apparent rate equal to the sum of these

reaction rates:

$$k = k_c + k_h \quad \text{eq. 2}$$

with  $k_c$  = rate constant of the chilling process, and  $k_h$  = rate constant of the heat process. Both rate constants  $k_c$  and  $k_h$  will, as for all chemical reactions, depend on temperature according to Arrhenius' law:

$$k_i = k_{i,\text{ref}} e^{\frac{E_i}{R} \left( \frac{1}{T_{\text{ref}}} - \frac{1}{T_{\text{abs}}} \right)} \quad \text{eq. 3}$$

where  $k_i$  is the rate constant of process  $i$  ( $c$  = chilling injury,  $h$  = high temperature deterioration),  $k_{i,\text{ref}}$  is the rate constant of process  $i$  at reference temperature  $T_{\text{ref}}$  ( $15^\circ\text{C}$ ),  $E_i$  is the energy of activation of that process, and  $T_{\text{abs}}$  is the actual absolute temperature in the experiment.

### Results

With these equations, the data of 20 species potted plants were analysed using multiple nonlinear regression, and the parameters estimated. In Figure 6-4 the acceptability is shown for six selected species as a function of storage time, in Figure 6-5 the acceptability of the same six species is shown as function of temperature. Except for two species, the explained variance ( $R^2_{\text{adj}}$ ) exceeded 90%, and was more than 95% in eight of the twenty species. The kinetic parameters of the model can directly be interpreted in terms of chilling and/or heat sensitivity. 15 species have a distinct value for the reference rate for the chilling process ( $k_{c,\text{ref}}$ ) while five species have no distinct value for that parameter. These results are in agreement with the generally accepted behaviour for these species.

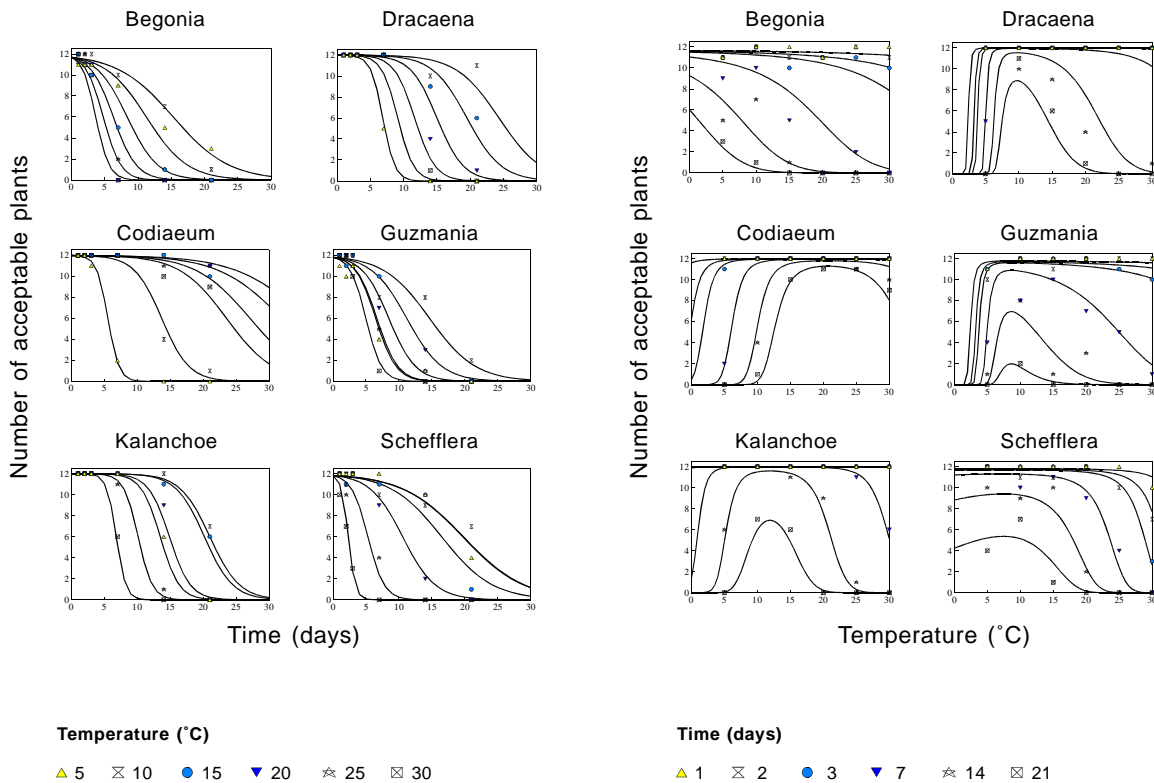


Figure 6-4 Measured (symbols) and simulated (lines) acceptability of six selected potted plant species versus storage time for the six experimental storage temperatures.

Figure 6-5 Measured (symbols) and simulated (lines) acceptability of six selected potted plant species versus temperature for the six experimental storage times.

From Figure 6-5 it is clear that the duration of storage in darkness has a pronounced effect on the apparent rate of decrease in acceptability: the longer a species has been stored or transported in darkness, the narrower the optimal temperature region becomes, the faster the loss in acceptability becomes, assuming some plants are still saleable, and the more pronounced differences due to temperature become. A practical consequence is that more care should be taken in selecting the optimal transport condition for transportation of longer duration.

From the model formulation it follows how the initial condition of the potted plants ( $N_0$ ) will affect the maximal acceptable transportation period.  $N_0$  also reflects the differences obtained in experiments (e.g. replications) with batches with apparently identical starting quality. The more  $N_0$  deviates from the maximum value  $N_{max}$ , the sooner the plants will become unacceptable at any temperature. In Figure 6-6 an example is given for the species *Dieffenbachia*, stored at 20 °C with an initial number of acceptable potted plants ranging from 11.94 to 11.999 on a total number of 12 plants in each batch. As can be seen, a small difference in initial vitality ( $N_0$ ) has a marked effect on the change in time of the acceptability when near the maximal possible count (12), and a far lesser effect at lower conditions of initial vitality.

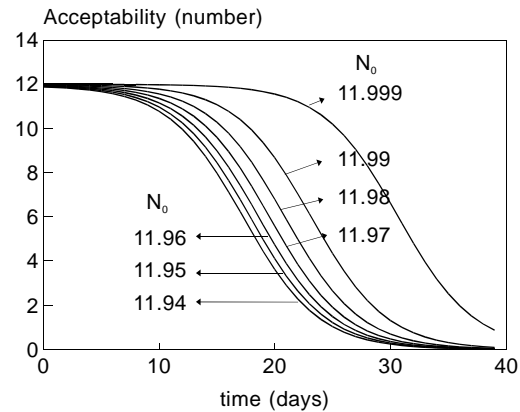


Figure 6-6 The effect of initial condition (vitality) at 20 °C for species *Dieffenbachia*.

The dynamic model allows calculation of the effects of storage in darkness on the acceptability as a function of variable temperature during storage. This provides a tool that allows wholesalers of potted plants and transport companies to calculate how long and within what temperature limits a product can be transported without adversely affecting its acceptability. It also allows insight into the optimum temperature and time for simultaneous transportation of several species of potted plants, with the quality of the most sensitive product as the limiting factor.

### **Keeping quality of perishable produce**

The effect of changing levels of quality criteria applied by consumers or users, cannot be taken from the type of model like used in the potted plant example, as it is based on data gathered from experiments using exclusively fixed acceptability limits. To develop models that include variable quality criteria, we have to search deeper into the mechanisms involved in quality decay. In the model on keeping quality (Tijskens 1995, Tijskens & Polderdijk 1994d, 1996a), these aspects are worked out for the four most commonly occurring mechanisms. All four mechanisms resulted in the same generic formulation of keeping quality show in eq. 4:

$$KQ = \frac{KQ_{ref}}{\sum_{i=1}^N k_i} \quad \text{eq. 4}$$

where  $KQ$  is the keeping quality,  $k$  is the rate constant for each of the occurring quality decaying processes  $i$  and  $KQ_{ref}$  is the keeping quality at reference temperature.

Keeping quality may be defined as the **time** the product's quality **remains acceptable** during storage or transport. Shelf life may be defined as the keeping quality at standardised conditions. The definition of acceptability, that is which product properties are included and which quality limits are actual applied, remains rather undefined and changes from produce to produce, from consumer to consumer, from situation to situation.

*Effect of quality limits*

To be flexible and generic, the quality limit, applied by a consumer or group of consumers, has to be included in the model formulation. Based on an assumed exponential mechanism as an example for the quality attribute(s) involved, the model on keeping quality is explained and the effects of different limits and initial qualities are shown. Other mechanisms are of course possible, with consequently a different relation for the limit applied.

$$KQ = \frac{\ln\left(\frac{Q_0}{Q_l}\right)}{\sum_{i=1}^N k_i} \quad \text{eq. 5}$$

where  $Q$  is the quality of the produce, indices 0 at initial time,  $l$  the limit value. The relation deduced for keeping quality of perishable products is shown in eq. 5. We see that (for this exponential mechanism of quality decay) the effect of different quality limits on the obtained keeping quality is a logarithmic one. This signifies that the change in keeping quality for a change in quality limit  $Q_l$  is lower at higher limits. It also signifies that the same effect can be obtained by increasing the initial quality by, e.g. 10% as by decreasing the quality limit by 10% and that with increasing the confidence of the consumer in your product instead of losing it.

In Figure 6-7 an example is given of the effect of (nationally) changing quality limits during international transport of cauliflower. Each time a border is crossed the quality limit changes to lower a level with an apparent increase in remaining keeping quality as a consequence. The quality of the product itself is of course not affected by this process. It takes only a longer time to reach the newly applied quality limit.

*Temperature effects*

The effect of temperature can again, as for the model on potted plants, be (at least) twofold ( $N=2$  in eq. 5). Most products of moderate origin are only susceptible to deterioration at higher temperatures. Most products of tropical or subtropical origin, however, are also highly sensitive to deterioration at low temperatures (chilling injury). This different behaviour is reflected in the equation by the sum of reaction rate constants in the denominator where  $N$  stands for the number of processes involved (or the number of attributes that can be limiting). These reaction rate constants again depend on temperature according to Arrhenius' law (eq. 3).

In Figure 6-8 an example is given for the behaviour of keeping quality for paprika (bell pepper) at constant storage conditions. The two temperatures driven processes can clearly be seen.

A discrete change in quality limit constitutes

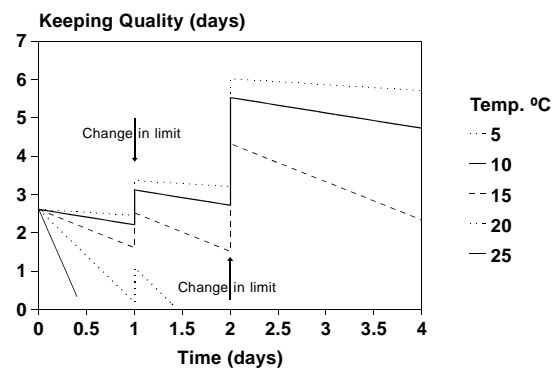


Figure 6-7 Effect of discrete changes in quality limit on the keeping quality of cauliflower at 5 constant temperatures (exponential decay).

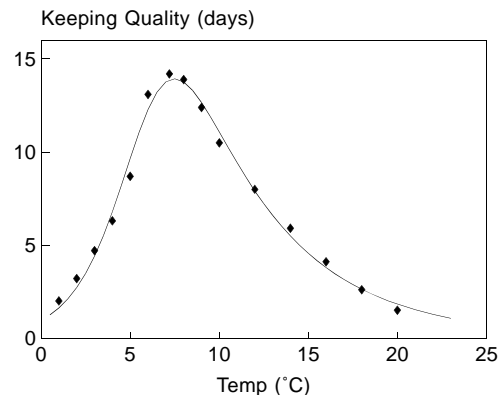


Figure 6-8 Keeping quality of bell peppers as a function of constant storage temperature.

an instantaneous change of the numerator in eq. 5, but still depends on temperature by the sum of reaction rate constants in the denominator. Temperature not only has an effect on keeping quality itself, changes in temperature also affect the importance of a change in quality limit. From Figure 6-7 it can be seen that the effect on keeping quality exerted by a changing quality limit is different for each temperature. The slower the processes of quality decay are, either at low temperature (no chilling injury, see 5 and 10 °C in Figure 6-7) or near the optimal temperature (chilling injury), the larger the effect of temperature becomes, due to the sum of rates in the denominator of eq. 5. This effect partly explains the difficulties encountered in practical conversion of keeping quality rules of thumb from one country to another.

#### *Effects of initial conditions and harvest time*

Lange & Cameron (1994) studied the effect of diurnal harvest time on the keeping quality of sweet basil. Analysis of the data revealed that the diurnal harvest time only affects the initial quality  $Q_0$ , without affecting the reaction rate constants. The initial quality could be described by a sinusoidal function, linked to the rise and fall of the sun during a day's period. Increasing the initial quality greatly increased the keeping quality and consequently the acceptability of the product. Again as for the potted plants, acceptability can be increased by increasing the initial intrinsic quality of the product without losing the consumer's confidence in the product by playing too close to the acceptability limit.

#### **Variance in batches**

Every batch of products consists of individuals. Despite the apparent identical stage of maturity, (external) quality and appearance, all these individuals are actually at different stages of maturity. The number of individuals in a batch that have to be acceptable for the batch to be acceptable (**batch quality limit**) depends on the preferences of the user(s) and the intended application. The first individuals in a batch that will become unacceptable will determine the acceptability of that batch. Inherently that are those individuals that are already more developed / mature to begin with. This fact puts a large emphasis on the type of distribution of the quality attribute in a batch of products with apparently an identical state of maturity.

With the recently developed very accurate techniques of quality measurements, especially the computer imaging techniques for measuring colour, and using the colour model similar to the model for the potted plants (see eq. 1), it becomes within reach to study the distribution of colour in a batch of products. Schouten & van Kooten (1998) reported on the type and change of the distribution of colour in batches of cucumbers. They found that the distribution of colour over the individuals in a batch is skew when the cucumbers are picked in a very early stage of maturity. The human eye can not discriminate between the colour of the individuals: they all are equally dark green. Upon maturation the distribution becomes less and less skew, but still dark green, and approaches the normal Gaussian distribution. The amount of skewness, that is how many cucumbers seem to be outliers with respect to the Gaussian distribution, depends among others, on the growing conditions.

In Figure 6-9 an example is given for batches of cucumbers, grown in two different plant densities and with two levels of nutrients. It is evident from this figure, that the skewness of the distribution will have a marked effect on the development of the batch rated as the acceptability. For batches with equal mean colour value, the batch with the largest skewness will first become unacceptable.

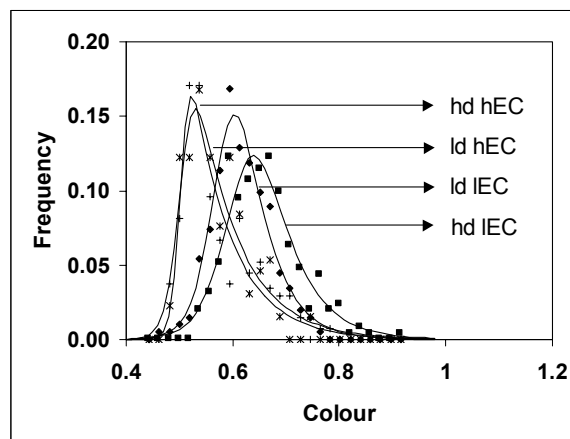


Figure 6-9 Distribution of green colour over the individuals of cucumber batches for two levels of plant density (ld and hd) and two levels of nutrients (IEC and hEC) during growing. (Courtesy ISHS).

How the distribution changes upon storage and further maturation, can be taken from Figure 6-10 where the background colour of batches of apples was measured individually during the ripening at several constant temperatures (unpublished, data M. Simčič, University of Ljubljana, Slovenia, analysis P. Konopacki, Research Institute of Pomology and Floriculture, Skierniewice, Poland). Clearly can be seen how the estimated distribution changes with storage time, and that they are somewhat different for apples of two successive harvest dates from the same orchard. What also can be taken from Figure 6-10 is that the apparent rate of colour development for already more mature individuals, is faster than for the mean of the batch, with on his turn is faster than the less mature individuals in the same batch. The same difference in apparent rate of maturation of batches of perishable products was reported by Tijssens & Wilkinson (1996c).

So, in conclusion, the mean value of different batches can be the same, the skewness of the distribution and the rate of development will determine how soon that batch will become unacceptable.

## Conclusions

The fact that all the batch acceptability of all the studied potted plants and that the keeping quality of all fruits and vegetables comply with the same models enhances their validity. It implies that a generic approach (one model for all potted plants tested, one model for keeping quality) is valid.

The same increase in acceptability and in keeping quality can be obtained by increasing the initial quality of the product as by a decrease in quality limit, however, without endangering the confidence of the consumer.

It is possible to combine the behaviour of intrinsic product properties, varying initial quality, variable quality limits and consumer behaviour into mathematical models. These models greatly enhance the understanding of the complex combined behaviour of acceptability.

Problem decomposition is a very valuable tool to develop mathematical models and descriptions of complex behaviour in living materials. This makes it even possible to describe mathematically the effect of rather ill or undefined entities like personally and regionally applied quality limits.

The skewness of the distribution of quality attributes, in batches of products with the same average quality level, largely determines the keeping quality of that particular batch.

## How to design further research?

To get a better understanding of acceptability and keeping quality of perishable products, and a better feeling for the effects of differences between apparently identical batches, future research should be design along a new and extended road.

- First of all, the relations depicted between quality attribute, acceptability and keeping quality need more attention and validation to ensure their application in a generic fashion. Can we really rely on intrinsic properties as sole descriptors of product

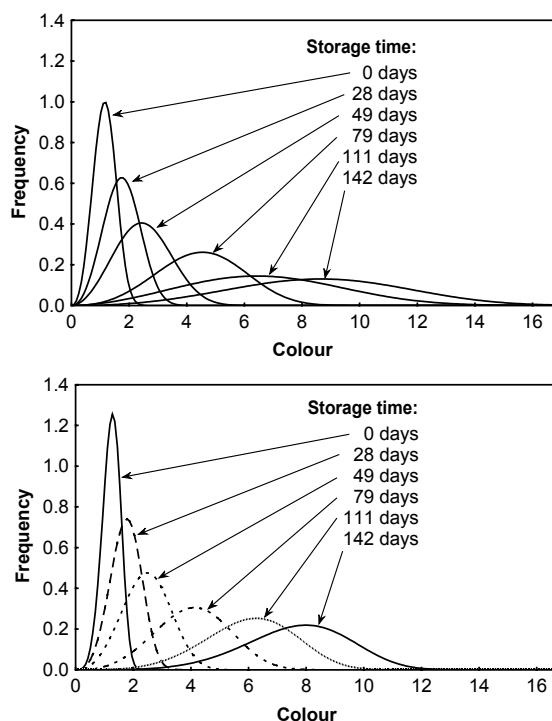


Figure 6-10 Distribution of colour of apples measured as a-value (green/red), for one orchard harvested at early (top) and mature (bottom) stage of maturity and stored up to 142 days at 4 °C

quality during the entire food chain? Is it really possible to decompose the quality problem into a problem of intrinsic and extrinsic properties and attributes?

- In view of the importance of the initial conditions in a batch, especially when the level of those initial conditions is very low and small differences have potentially a major effect, new and very sensitive measuring techniques should be developed to measure the appropriate quality attributes and quality properties. For colour of all kinds of fruits and vegetables, this technique is already available in the computer image analysis system. For quality attributes like firmness and amount of bacterial infection more sensitive and non-destructive techniques have to be developed.
- The effect of distributions of a quality attribute or product property in a batch needs to be studied in more detail, and the possibilities for practical applications have to be assessed.
- The design of research experiments should focus on non-destructive techniques that make it possible to reuse and reassess the same individual fruit at any later time. Individual monitoring and its administration should receive much more attention in practical research, to fully use its potential for chain management and optimisation.

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## **Part 3**

# **QUALITY POSTHARVEST**

## Introduction

We now have established the framework of quality, how it can be made as objective as possible. We have established the framework of modelling, how to apply the technique of problem decomposition and making the full use of rules of discipline and laws of nature. Now we can make a start towards constructing models for describing and understanding the quality of agricultural produce. However, one more very important part of all changes in living material merits a separate approach and separate attention: enzyme systems.

Almost all conversions and reactions inside living material are made possible by the action of enzymes or enzyme systems. Although enzymes exist in a wide variety of structures and exerted action, some properties of enzyme systems are generic and comparable to all. All enzymes work faster at higher temperatures. At some high temperature however, the exerted action decreases, most often quite rapidly with increasing temperatures. These activity profiles and the so-called optimal temperature can be found for a wide variety of enzymes in textbooks and reference books.

In the traditional line of thinking, describing the observed behaviour, modellers have tried to formulate empirical functions and relations (mostly polynomials) applicable over the complete range of temperature. Building up models based on a thorough decomposition of the problem and applying the fundamental rules of chemical kinetics, the behaviour can be analysed, described and modelled quite easily and neatly. What is really going on at “lower” temperatures, increasing the activity of enzymes with increasing temperatures, is a mere consequence of the Arrhenius dependence on temperature of the conversion reaction itself. At higher temperature, the enzyme itself starts to denature, thereby decreasing the molecular amount of available and active enzyme. The rate of conversion of substrate to product itself keeps on increasing with increasing temperature. So, decomposing the problem into two separate processes, points directly the way to solve the problem in a generic and fundamental manner. The complete model can be represented by the following mechanism:



as it is developed and shown in chapter 2, eq. 1 and chapter 3 eq. 1. In summary, the apparent activity of any enzyme as a function of temperature can be represented by the overall equation (eq. 2).

$$\text{Act} = \text{Enz}_0 \cdot k_s \cdot e^{-k_d \cdot t} = \text{Act}_0 \cdot e^{-k_d \cdot t} \quad (\text{eq. 2})$$

From the representation in Figure 7-1, it is clear that the so-called optimal temperature depends on the time during which the assay is conducted or during which the heat treatment is applied. Depending on the value of the rate constants at reference temperature and the values of the activation energies, the overall shape of the curves can virtually take any form.

Of course, for more complex mechanisms, like e.g. iso-enzyme systems, the expression will be more complicated. The general line of reasoning, however, remains the same: two processes describe the activity of each single enzyme species, an activation by temperature (Arrhenius relation) and a temperature-time decay mechanism (combined first order kinetics and Arrhenius relation).

Another important aspect of enzyme systems is the so-called Michaelis Menten approach. This approach is based on the assumption that an intermediate complex is formed between the substrate and the enzyme at the active site of the enzyme, and that the formation of the active complex is in steady state conditions: the active complex is more rapidly formed than the conversion into product. This approach leads to the very well known Michaelis Menten equation:

$$\frac{dS}{dt} = -\frac{k_s \cdot \text{En} \cdot S}{K_m + S} \quad \text{eq. 3}$$

In enzymology the value of this equation is acknowledged. However, on many occasions this famous Michaelis Menten equation has been criticised to be inapplicable in almost any real life situation. One should however bear in mind that the ultimate value of this deduction does not reside in the formulation of the equation but completely in the line of reasoning followed to arrive at that equation. So, for real life systems, we have to decompose the actual situation and the actual problem into the constituting elements (e.g. in iso-enzyme systems) and then apply that line of reasoning to each active species in the system.

Since enzyme systems are so powerful and absolutely necessary for living matter, examples of this line of reasoning, applied to specific and particular conditions will be found in almost any of the chapters in this work in various degrees of complexity and difficulty.

In chapter 7, a more generic formulation is developed to show that various and different apparent behaviour of real time enzyme systems can be described in a complex but still remarkably simple overall mechanism. Similar reaction mechanisms have been proposed and tested (Polakovič and Bryjak, 2002) under strictly dynamic conditions, without applying steady state assumptions. The model is applied to specific enzymes like pectin methyl esterase (PE) and peroxidase (POD), comprising not only the above-indicated activation and denaturation but also a conversion from an inactive enzyme precursor into active enzyme. The model formulations not only describe the behaviour of enzyme activity during storage and processing, but allowed also the analysis the raw measuring data to be analysed entirely, including the increasing non-linearity at higher denaturation levels. The model was applicable in two consecutive seasons (PE), not only with respect to the model structure, but also with the same values for the kinetic parameters.

The process that governs all controlled atmosphere storage and modified atmosphere packaging is the respiration of living material. The ongoing processes are for the majority of agricultural products and in the majority of circumstances roughly the same. Of course, each particular product will have its own particular initial state and its own particular values for the kinetic constants. In chapter 8, a generic model is presented and described covering the gas exchange including respiration and fermentation in anoxic circumstances for several products.

In chapter 9, a model on chilling injury, induced at (too) low temperatures, but on many occasions only becoming visible at higher post-chilling storage temperatures is developed. In this model, not only the intrinsic product properties (membrane damage, free radical levels, free radical scavenging activity) are described, but also the effect these levels have in the evaluation and acceptability of the product by target consumers.

Based on the theory developed in Part 2, the quality assigned to an agricultural product is almost completely based on the product properties at hand. The only time a 'standard' consumer is needed is when the quality attributes need to be defined in terms of importance and occurrence. This 'definition' is almost trivial for standard horticultural produce and for standard human consumers. Over and over again in literature, a report on research quality of fruits and vegetables starts with a survey of the quality attributes important to consumers and users throughout the food chain. Of course, the fine-tuning of attributes to a particular segment of the consumer market will be slightly different from case to case and from situation to situation. More often than not, this fine-tuning both for a particular segment of consumers as for a particular fruit or vegetable, is just a mere variation on the same attributes over and over again.

The standard most often used external quality attributes for the majority of fruits and vegetables are:

- firmness
- colour
- absence of defects
- shrivelling and water loss

Lately the internal quality attributes gain more and more importance for the consumer and hence for the whole chain. These attributes comprise:

- taste (sweet, sour)
- aroma and flavour
- juiciness
- crispness
- absence of fibrousness
- vitamins
- health promoting compounds

The presence of texture related attributes, both in the external (firmness, shrivelling) as in the internal attributes (juiciness, crispness, fibrousness), is overwhelming (Bourne 2002). This is already a strong indication of the importance people attribute to texture properties. It also signifies that textural attributes together with colour are some of the few quality attributes that a consumer can use to evaluate produce quality when buying agricultural produce. Both attributes (colour and firmness) are frequently used as indicators for the overall quality, and build up the expectations at the consumers or users.

The colour of agricultural products evaluated by visual inspection is most of the time quite straightforward. How red is a tomato, how green a cucumber are not the most difficult assessments to be performed. Even the existing variation within one individual or within a batch of one commodity can be and is being used as an indicator for ripeness, freshness, keeping quality and overall quality. The colour of products is more or less directly related to the amount of colouring compounds present in the product. Examples of the modelling of colour behaviour during storage and processing can be found in Tijskens (1994), Tijskens and Evelo (1994), Tijskens et al. (2000), Tijskens et al. (2001), Tijskens et al. (2001 see chapter 12) and Schouten et al. (2002).

For textural attributes the relation with constituting compounds in the product is not so evident. Van Dijk and Tijskens (2000b, see also chapter 11) discerned seven possible sources of texture. Five of them are of chemical nature, two are of physical and structural nature. That already indicates the complexity of these textural attributes. In a recent survey Luyten (2003) made an inventory how sensory textural attributes relate to physical/chemical properties of the produce. This survey shows clearly that the interactions between different physical/chemical properties are highly complex, but describe qualitatively the existing relations. In other words for textural attributes the relations with physical and chemical properties of the product are not at all clear at the moment. That uncertainty is directly reflected in the difficulties encountered in setting up plausible and appropriate kinetic mechanisms to describe the important processes that make up in the long run the behaviour of texture.

In chapter 10, several fundamental processes concerning firmness decay by enzymatic action and retaining firmness by reducing the respiration have been combined in an application on firmness of Elstar apples. Polygalacturonase (PG) and pectin methyl esterase (PE) are known to affect firmness behaviour during postharvest storage (Stolle-Smits 1998). On a qualitative level, the theory on the interactions between PG and PE is clearly established: PG can only decrease the degree of polymerisation where and when PE has de-esterified the pectin backbone. On a quantitative level, this theory is very hard to prove. Only sparsely data are available that confirm this theory (Majumber and Mazumdar 2002). Since both these enzymes act on pectins as substrate, only that part of firmness that can be traced back to this chemical component of firmness, like e.g. in hard fruit (apples, pears) root products (carrot, turnip, kohlrabi) and vegetables (green beans), will show this type of behaviour. But other sources of firmness generation and firmness decay (cell turgor and tissue tension, water loss, structural cellulose related firmness, etc.) will be important at various levels in even these products.

Further examples of process oriented models on firmness behaviour, of firmness as affected by action of different enzymes and of the behaviour of firmness related enzymes can be found in Tijskens and Schijvens (1987), Tijskens and Rodis (1997), Tijskens et al. (1997a), Tijskens et al. (1997b), Rodis et al. (1997), Tijskens et al. (1998), Tijskens et al. (1999), Tijskens et al. (1999b), Tijskens and van Dijk C. (2000), Van Dijk and Tijskens (2000a,

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**7**

**GESSI:**

**A generic enzyme system on stimulation and  
inactivation during storage and processing**

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Lecture delivered at the Second International Conference *AGRI-FOOD QUALITY II*. 22-25  
April 1998, Turku, Finland. In Hägg M., Ahvenainen R., Evers A.M., Tiilikkala K. (eds.) p.  
81-86, Royal Society of Chemistry, Cambridge, UK.

## Introduction

The phenomena, observed in living plant parts as response to their environment, either natural or artificial, are so numerous, scientists have already been studying the behaviour of food as long as science exists. Nature has almost unlimited resources of chemical and biochemical compounds, and can play with different levels of those compounds to achieve its goals: prolonging the life span and increase the distance a plant can proliferate. The number of processes that nature uses to reach this overwhelming variability in observed phenomena, however, is in a generic sense rather limited: with for example only the thirty-odd chromosomes present in humans, a uniqueness among all men, living and deceased, is obtained. The same generic approach can be used to describe the phenomena observed with the quality of our food.

## Enzymes in living plants

During the life span of agricultural foods, all kinds of enzymes are active. These enzymes serve the plant to stay alive by fulfilling the natural physiological purpose that plant part has for the plant as a whole. Even during processing operations, like blanching and sterilisation, these enzymes may play a major part in the observed changes in what human like to call food quality.

During research, conducted in the framework of a EU-AIR program on textural behaviour during processing, a model was developed that describes the changing level of pectin methyl esterase (PE), polygalacturonase (PG) and peroxidase (POD) in several products (peaches, potatoes, carrots, green beans) and the effect of blanching temperatures on the activity (heat denaturation) and exerted action of those enzymes. The observed behaviour and exerted action were very different for these enzymes and for the different products. This overall mechanism, however, has been applied not only to the aforementioned enzymes and products, but also to completely different enzymes systems like lipase, lipoxygenase and aroma forming enzymes in a number of products like rapeseed, green beans and bell peppers.

## Enzyme activity

When assaying enzyme activities, usually the amount of substrate converted into some product is measured at some standard temperature and expressed as activity. What really is measured is the rate of conversion at that temperature. If we reflect on the Michaelis Menten equation (eq. 1), we see that the activity of an enzyme, expressed in such a way, is a combination of the specific activity at that temperature and the (unknown and unmeasurable) concentration of the enzyme. The specific activity will depend on temperature, presumably according to Arrhenius' law. What this combination will do at higher temperatures, where a first order exponential inactivation or denaturation of the enzyme occurs is shown in Figure 7-1.

$$\frac{\partial S}{\partial t} = - \frac{k_s \text{Enz} S}{K_m + S}$$

eq 1

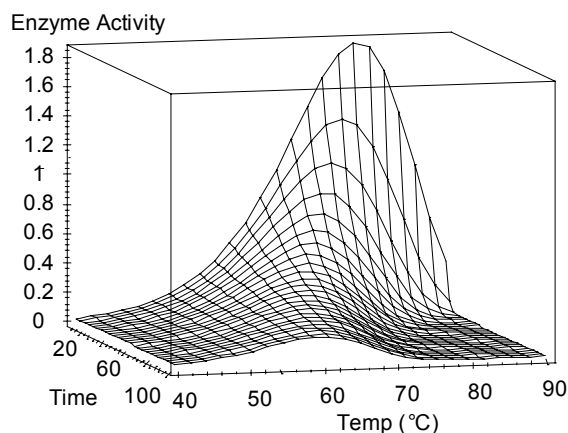


Figure 7-1 Denaturation of an arbitrary enzyme

So, in the remainder of this chapter, we have to remind that all activities are expressed as the activity, at a standard constant temperature, of the enzyme remaining after a heat treatment at different temperatures and times.

## Specific model formulations

For POD in peaches, carrots and potatoes a bound and a soluble enzyme was found, both of which can be active (Tijskens et al. 1997c). The mechanism proposed is shown in eq. 2. Also for PE in peaches, carrots and potatoes a bound and a soluble enzyme was found, again both active (Tijskens et al. 1997a, 1997b, 1999). The same mechanism as proposed for POD is applicable (eq. 2).



The same mechanism was found to be applicable to PE in several cultivars of green beans (A&F, unpublished).

The most prominent aspect of the mechanism for PG in peaches (Tijskens et al. 1998) was the conversion from an inactive precursor into an active configuration (eq. 3). This could be regarded as a turnover normally present in enzyme systems of living plants.



In each of these mechanisms denaturation (index d) occurs into an inactive or at least less active configuration, either by heat treatment or by senescence. The denaturation of lipases was studied in rape seed oil as a function of heat treatment by steam or microwave (Ponne et al. 1996). Again, two iso-enzymes were found to exist, this time without a conversion from the one species into the other. A third iso-enzyme resisted denaturation completely, thereby allowing for a residual activity after heat treatment. The enzyme system related to aroma development in bell peppers after heat also showed susceptibility to heat denaturation (Luning et al. 1995).

## Generic model formulation

All these models, built for each combination of enzyme type and product, always seem to be a special case of a generic underlying overall mechanism. This mechanism includes:

- generation of active species from a precursor
- conversion from one active configuration to another one (iso-enzymes)
- senescence induced loss of activity (natural turnover)
- heat induced denaturation of both active species

The generic mechanism is schematically presented in Figure 7-2. This mechanism can describe an almost infinite number of combinations of enzyme systems, living plant parts, and applied temperature scenarios. Although the apparent enzyme behaviour,

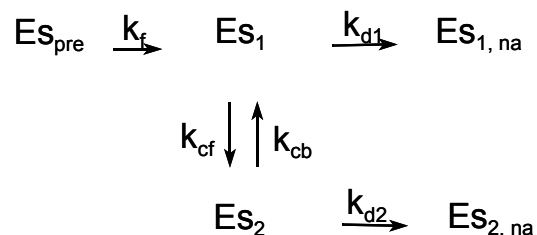


Figure 7-2 Generic model formulation

measured or observed, is different for different combinations of products and enzymes and for different batches of products, the mechanism remains unchanged. Furthermore, the kinetic parameters (the reaction rate constants at reference temperature and the energies of activation) seem to remain unchanged for different batches of products. They can (probably) be regarded to be specific for a species or cultivar. This opens a wide alley to application of this model and the connected kinetic parameters for situations occurring in practice (Tijskens et al. 1996).

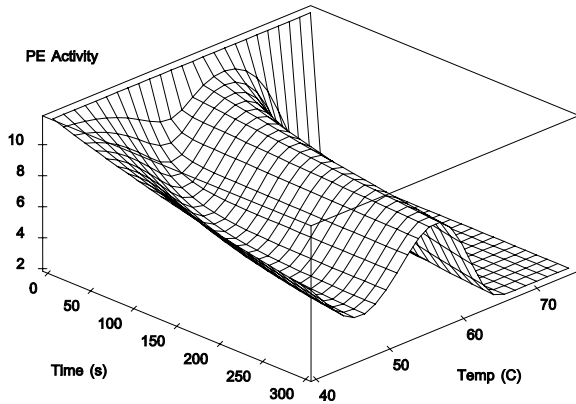


Figure 7-3 PE activity in peaches during blanching (season 1994)

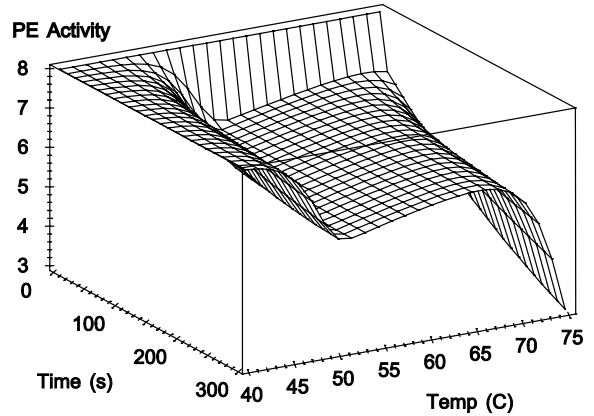


Figure 7-4 PE activity in peaches during blanching (season 1995).

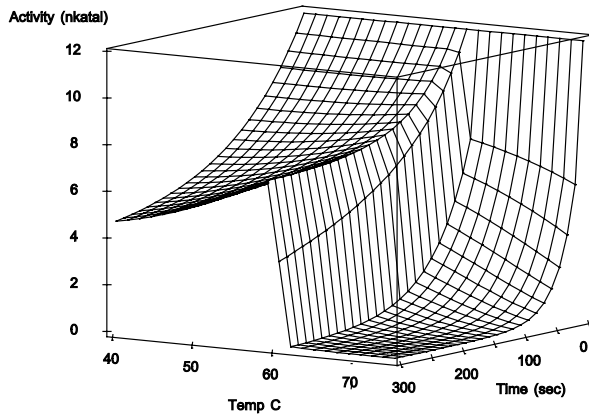


Figure 7-5 PE activity in peaches during blanching (1994, combined analysis).

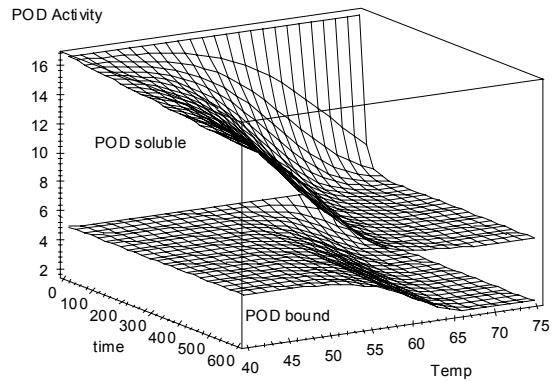


Figure 7-6 POD activity, bound and soluble, in peaches during blanching

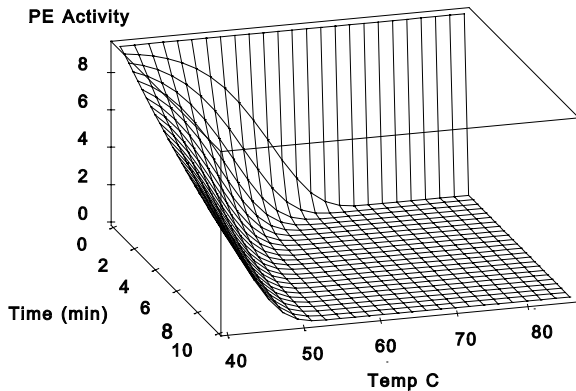


Figure 7-7 PE activity in carrots during blanching

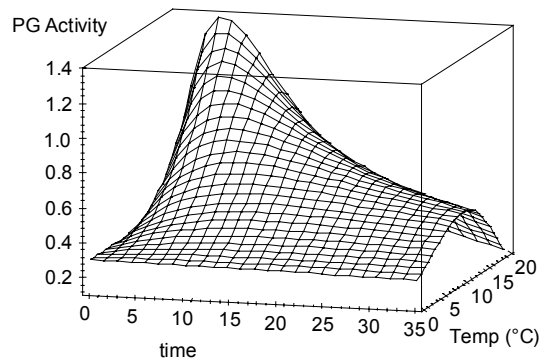


Figure 7-8 PG activity in peaches during storage

In Figure 7-3 to Figure 7-10 some three dimensional examples are given to elucidate how differently the observation on enzyme activity can be in different circumstances of product, enzyme type, seasonal variation, all based on the same generic system and the species specific parameters. The parameters were obtained by analyses of appropriate sets of data, and based on the specific model formulations, derived for the combination of product and enzyme type. In Table 7-1 the value of the parameters, used in the graphs and the demonstration, are shown without further details of analysis.

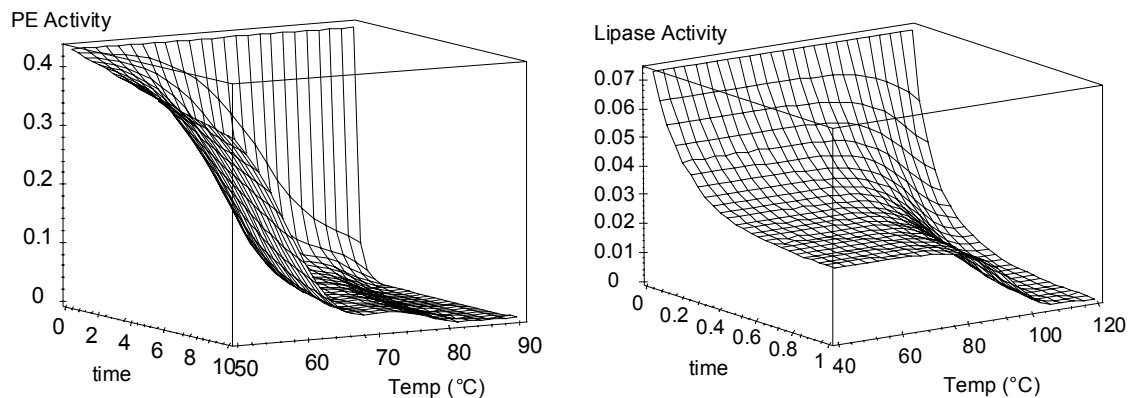


Figure 7-9 PE activity in green beans (cv. Masai) during blanching. Figure 7-10 Lipase activity in rapeseed oil during heating in steam and microwave.

## Enzyme classification

When iso-enzymes can be converted into one another the molecular mass may change (cleavage of the protein carrier chain, unbinding to matrix structure), the stereo configuration may change (internal cross links, hydrogen bonds or internal chelating), a change in shielding of the active site may occur (sometimes more, sometimes less), but the general structure of the active site remains roughly the same.

To stress the similarities in active sites of convertible iso-enzymes, the generally accepted classification of iso-enzymes, mainly based on molecular mass (kDaltons) and iso-electric focussing point, all of which are aimed at the properties of the protein carrier, should be extended to include a specification of the active site itself, irrespective of the protein chain they are tied to, and the magnitude and configuration of the protein chain itself. A classification of the obtained model parameters, especially the kinetic parameters connected to activity, conversion and denaturation, could serve as a start and a guideline for this distinction in iso-enzyme type.

An example of this approach is the lipoxygenase activity in green beans. The activity of the extracted enzyme was roughly 20 times lower as in the intact beans, either by change of stereo-configuration or by lesser accessibility. The denaturation properties (rate constant of denaturation and energy of activation) were, however, in both cases exactly the same.

## Acknowledgment

This study was conducted within the framework of the EU AIR project AIR1-CT92-0278 on The biochemistry and archestructure of fruit and vegetable tissue as quality predictors for optimising storage and processing regimes: Basic research leading to applicable models and rules, partly financed by the European Union.

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7: Gessi: A Generic Enzyme System

Table 7-1 Results of the statistical analyses using nonlinear regression from different enzyme system in different products

<b>Gessi model Parameter estimates</b>																		
Product	Cultivar	Enz	Year	Tref	$E_{p0}$	$E_{s10}$	$E_{s20}$	$E_{sfix}$	$k_{ref}$	$E_f/R$	$k_{c1ref}$	$E_{c1}/R$	$k_{cbref}$	$E_{cb}/R$	$k_{d1ref}$	$E_{d1}/R$	$k_{d2ref}$	$E_{d2}/R$
Carrot	Bak	PE	1994	60	0	1.473	12.5	0	0	0	1.64	14535	0	0	0	0	0.04407	19318
Peach	Andross	PE	1994	60	0	8.5	1.4	2.07	0	0	1.2	57000	0	0	0.1352	20962	1.954E-3	41833
Peach	Andross	PE	1995	60	0	8	0	0	0	0	1.2	57000	0	0	3.46E-01	52995	4.02E-6	50490
Peach	Andross	PE	1994	60	0	9.2	2.24	0	0	0	0.01027	8031	0	0	0.008	500	0.000391	68143
Peach	Andross	PE	1995	60	0	4	4.88	0	0	0	0.01027	8031	0	0	0.008	500	3.33E-7	68143
Potato	Bintje	PE	1994	60	0	1.823	5.5	0	0	0	0.255	10960	0	0	0	0	0.06506	17809
Gr. Beans	Flotille	PE	1995	60	0.884	0.4315	0	0	0.00215	28518	0	0	0	0	0.1357	18941	0	0
Gr. Beans	Masai	PE	1995	60	0.003988	0.14892	0	0	0.001623	32938	0	0	0	0	0.00827	25932	0	0
Peach	R Haven	PG	1994	10	0.3	8.37	0	0	0.0173	13646	0	0	0	0	0.179	5671	0	0
Peach	R Haven	PG	1995	10	12.52	122	0	0	0.0664	6266	0	0	0	0	0.269	0	0	0
Peach	R Haven	PG	1994	10	2.276	0.235	0	0	0.0638	11711	0	0	0	0	0.1023	0	0	0
Peach	R Haven	PG	1995	10	40.91	18.17	0	0	0.0638	11711	0	0	0	0	0.1023	0	0	0
Peach	Andross	POD	1994	60	0	3.5	10	0	0	0	0.0013	28541	0	0	0	0	0.00844	18026
Carrot	Bak	POD	1994	60	0	40	0	0	0	0	0	0	0	0	9.6E-04	14249	0	0
Potato	Bintje	POD	1994	60	0	15	0	0	0	0	0	0	0	0	8E-07	51034	0	0
Gr. Beans	Masai	POD	1996	60	0	21.816	0	0	0	0	0	0	0	0	0.01059	26026	0	0
Rape Seed		Lip	1993	90	0	0.039	0.025	0.016	0	0	0	0	0	0	0.096	5900	0.124	0
Rape Seed Oil		Lip	1993	90	0	0.048	0.025	0.007	0	0	0	0	0	0	5.27	0	0.46	18041
Bell pepper	Hexenol	unk	1993	80	0	1	0	0	0	0	0	0	0	0	0.1957	6140	0	0
Cucumber	El Trans	unk	1998	20	0	1	0	0	0	0	11.5	18500	1	0	0	0	0	0





**8**

**A dynamic and generic model of gas exchange**

**of respiring produce:**

**the effects of oxygen, carbon dioxide and temperature**

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Published in *Postharvest Biology and Technology* **14** (1998) 335–349

## Abstract

A generic model is developed that describes rates of respiration and fermentation, in response to temperature and environmental gas conditions (oxygen and carbon dioxide). The mathematics of the model are based on Michaelis–Menten kinetics. Respiration is simplified as the effect of one enzymic reaction, inhibited by its own product, carbon dioxide. Both competitive and uncompetitive inhibition are incorporated. Fermentation is modelled including the competitive inhibition of fermentation by both oxygen and carbon dioxide. Temperature dependence is introduced using Arrhenius equations for the maximum rates of oxygen consumption and carbon dioxide production. The model is generic in nature: it is applicable to different types of products (apple, chicory and tomato) at different conditions (in terms of O<sub>2</sub>, CO<sub>2</sub> and temperature) and for different types of inhibition. This fitness is an indirect validation of the assumptions on which the model is based.

**Keywords:** Apple; Chicory; Fermentation; Gas exchange; Inhibition; Mathematical model; Respiration; Tomato

## Introduction

The success of controlled atmosphere storage and modified atmosphere packaging depends on a reduction of the metabolic rate of the product (Ulrich, 1975; Burton, 1978). Commonly a combination of low O<sub>2</sub>, high CO<sub>2</sub> and low temperature is applied (Henig, 1975; Kader et al., 1989; Riquelme et al., 1994). Individual effects of O<sub>2</sub>, CO<sub>2</sub> and temperature may be additive and the total effect can be greater when these factors are combined (Ulrich, 1975; Zagory and Kader, 1988; Kader et al., 1989). The optimal condition for storage strongly depends on the metabolic characteristics of the specific product (Kader et al., 1989; Cameron et al., 1995). A good understanding of the dynamics of the metabolic rate is essential for the optimization of controlled atmosphere storage and modified atmosphere packaging.

One early study of the integral effects of O<sub>2</sub>, CO<sub>2</sub> and temperature on gas exchange was reported by Fidler and North (1967). Interpretation of such complex results on gas exchange as a function of O<sub>2</sub>, CO<sub>2</sub> and temperature can be considerably improved by using a mathematical model. Several attempts have been made to model gas exchange either by empirical models (Jurin and Karel, 1963; Hayakawa et al., 1975; Yang and Chinnan, 1988; Cameron et al., 1989; Raghavan and Gariepy, 1989; Talasila et al., 1992) or strongly simplified models using, for instance, a single Arrhenius equation (Mannapperuma et al., 1989; Mannapperuma and Singh, 1994). A more fundamental approach was applied by Chevillotte (1973) who introduced Michaelis–Menten kinetics to describe respiration. Lee et al. (1991) included uncompetitive inhibition by CO<sub>2</sub> and tested the model on cut broccoli.

Peppelenbos and Van't Leven (1996), evaluated four types of inhibition for modelling the influence of CO<sub>2</sub> levels on O<sub>2</sub> consumption of fruits and vegetables as compared to no influence of CO<sub>2</sub>. They introduced an equation describing the O<sub>2</sub> consumption rate ( $V_{O_2}$  in mmol kg<sup>-1</sup> s<sup>-1</sup>) as inhibited by CO<sub>2</sub> both in a competitive and in an uncompetitive way. This combined type of inhibition of O<sub>2</sub> consumption was formulated as:

$$V_{O_2} = \frac{V_{m_{O_2}} \cdot [O_2]}{K_{m_{O_2}} \cdot \left(1 + \frac{[CO_2]}{K_{m_{CO_2}}}\right) + [O_2] \cdot \left(1 + \frac{[CO_2]}{K_{m_{i_{CO_2}}}}\right)} \quad \text{eq. 1}$$

where [CO<sub>2</sub>] and [O<sub>2</sub>] are concentrations (%),  $V_{m_{O_2}}$  the maximum O<sub>2</sub> consumption rate (mmol kg<sup>-1</sup> s<sup>-1</sup>),  $K_{m_{O_2}}$  the Michaelis constant for O<sub>2</sub> consumption (%),  $K_{m_{CO_2}}$  the Michaelis constant for the competitive inhibition of O<sub>2</sub> consumption by CO<sub>2</sub> (%) and  $K_{m_{i_{CO_2}}}$  the Michaelis constant for the uncompetitive inhibition of O<sub>2</sub> consumption by CO<sub>2</sub> (%). The combined type of inhibition is a comprehensive and flexible formulation that, depending on the values of  $K_{m_{CO_2}}$  and  $K_{m_{i_{CO_2}}}$ , can describe all generally distinguished types of inhibitions on the rate of O<sub>2</sub> consumption (competitive, non-competitive and uncompetitive inhibition of O<sub>2</sub>

consumption by CO<sub>2</sub>; Chang, 1981) including the case of no inhibition (Table 8-1). The respiration model without inhibition by CO<sub>2</sub> has been successfully applied for several products (apple, Andrich et al. (1991); blueberry, Cameron et al. (1994); red raspberry, Joles et al. (1994); cauliflower, Ratti et al. (1996)). The non-competitive inhibition was applied by Peppelenbos et al. (1993) to fresh mushrooms and by Song et al. (1992) to blueberry. Renault et al. (1994) applied the model of uncompetitive inhibition from Lee et al. (1991), which describes O<sub>2</sub> consumption, to describe CO<sub>2</sub> production, thereby assuming a respiration quotient of 1 and neglecting a possible contribution of fermentative CO<sub>2</sub> production. According to Peppelenbos et al. (1996), CO<sub>2</sub> production ( $V_{CO_2}$  in mmol kg<sup>-1</sup> s<sup>-1</sup>) results from both oxidative and fermentative processes simultaneously and can be described as:

$$V_{CO_2} = RQ_{ox} \cdot V_{O_2} + \frac{Vm_{CO_2(f)}}{1 + \frac{[O_2]}{Kmc_{O_2(f)}}} \quad \text{eq. 2}$$

where  $RQ_{ox}$  represents the respiration quotient (ratio between CO<sub>2</sub> production and O<sub>2</sub> consumption) for oxidative respiration,  $Vm_{CO_2(f)}$  the maximum fermentative CO<sub>2</sub> production rate (mmol kg<sup>-1</sup> s<sup>-1</sup>) and  $Kmc_{O_2(f)}$  the Michaelis constant for the competitive inhibition of fermentative CO<sub>2</sub> production by O<sub>2</sub> (%).

Table 8-1 The multiple faces of the formulation for the combined type of inhibition of O<sub>2</sub> consumption by CO<sub>2</sub> depending on the value of the parameters  $Kmc_{CO_2}$  and  $Kmu_{CO_2}$

Parameter value <sup>a</sup>	Resulting type of inhibition	Resulting formulation for $V_{O_2}$ <sup>b</sup>
$Kmc_{CO_2}=+\infty$ and $Kmu_{CO_2}=+\infty$	No inhibition: a simple between O <sub>2</sub> concentration and O <sub>2</sub> consumption	$\frac{Vm_{O_2} \cdot [O_2]}{Km_{O_2} + [O_2]}$
$Kmu_{CO_2}=+\infty$	Competitive: CO <sub>2</sub> competes with the substrate (O <sub>2</sub> ) for the same active site of the enzyme	$\frac{Vm_{O_2} \cdot [O_2]}{Km_{O_2} \cdot \left(1 + \frac{[CO_2]}{Kmc_{CO_2}}\right) + [O_2]}$
$Kmc_{CO_2}=+\infty$	Uncompetitive: CO <sub>2</sub> reacts with the enzyme-substrate complex	$\frac{Vm_{O_2} \cdot [O_2]}{Km_{O_2} + [O_2] \cdot \left(1 + \frac{[CO_2]}{Kmu_{CO_2}}\right)}$
$Kmc_{CO_2}= Kmu_{CO_2}$ (= $Kmn_{CO_2}$ )	Non-competitive: a one-to-one combination of competitive and uncompetitive inhibition	$\frac{Vm_{O_2} \cdot [O_2]}{(Km_{O_2} + [O_2]) \cdot \left(1 + \frac{[CO_2]}{Kmn_{CO_2}}\right)}$
<sup>a</sup> $Kmi_{CO_2}$ =Michaelis constant for inhibition of O <sub>2</sub> consumption by CO <sub>2</sub> (%); $l=c$ : competitive; $l=u$ : uncompetitive; $l=n$ : non-competitive. <sup>b</sup> $V_{O_2}$ =O <sub>2</sub> consumption rate (mmol kg <sup>-1</sup> s <sup>-1</sup> ); $Vm_{O_2}$ =the maximum O <sub>2</sub> consumption rate (mmol kg <sup>-1</sup> s <sup>-1</sup> ); $Km_{O_2}$ =Michaelis constant for O <sub>2</sub> consumption (%); $[CO_2]$ , $[O_2]$ : concentration (%).		

To prevent any confusion, the following definitions are used throughout this paper. Respiration is O<sub>2</sub> consumption ( $V_{O_2}$ ) and CO<sub>2</sub> production due to oxidative processes ( $RQ_{ox} \cdot V_{CO_2}$ ). Fermentation is CO<sub>2</sub> production from anaerobic processes  $V_{CO_2(f)}$ . Gas exchange involves both respiration and fermentation. Eqs. (1) and (2), describing the effect of O<sub>2</sub> and CO<sub>2</sub> on gas exchange, constitute the basis for the present model. The model is extended with a temperature dependence according to Arrhenius' law. A comparable approach, based on uncompetitive inhibition extended with a temperature dependence according to Arrhenius' law, has already been suggested by Tijskens (1996) but not fully validated. On the basis of the data presented here, the model is also extended with an inhibition of the fermentative CO<sub>2</sub> production by CO<sub>2</sub>. A comparable approach was used by Peppelenbos et al. (1998) to

describe gas exchange of mungbean sprouts. The aim of this research was not to model the complete biochemical pathways of respiration and fermentation, but to establish a generic model at the product level, based on a simplified interpretation of the underlying biochemical mechanism. In this paper, the developed model is fitted to sets of data describing gas exchange of apple, chicory and tomato, as a function of temperature and environmental gas conditions (O<sub>2</sub> and CO<sub>2</sub> concentration).

## Material and methods

### Products

Data on gas exchange were collected over several years within the framework of the EC-AIR MASTER project (modified atmosphere systems in varying temperature regimes; no. AIR2-CT- 1326) by Institute Nationale de la Recherche Agronomique (INRA, Montfavet, France; data on chicory and tomato), Istituto di Industrie Agrarie (University of Pisa, Italy; data on apple), and Verbond van Belgische Tuinbouwveilingen (VBT, Leuven, Belgium; data on apple, chicory and tomato). The products were purchased locally by the participants over the years 1994-1997. For apples (*Malus domestica* Borkh.), the cultivar 'Golden Delicious' was used. Chicory endives (*Cichorium intybus* L.) were the cultivars 'Monitor' and 'Diamant' while tomatoes (*Lycopersicon esculentum* Mill) were 'Maëva' and 'Trust'.

### Gas exchange measurements

Each of the participants used his own technique to measure gas exchange. INRA used a flow-through respirometer coupled with an infrared CO<sub>2</sub> analyser (Varoquaux et al., 1995). The University of Pisa used a flow-through respirometer coupled with a continuously monitoring gas chromatograph (Andrich et al., 1994). VBT applied head space analysis with gas chromatography. VBT monitored the change in O<sub>2</sub> and CO<sub>2</sub> as a function of time on closing the containers, after a flushing period with gas of the desired composition. Gas exchange was measured at different temperatures (1, 6, 11, 16 and 21°C for apple and chicory and 8, 13, 18, 23 and 28°C for tomato), O<sub>2</sub> levels (0, 3, 10 and 21% O<sub>2</sub>) and CO<sub>2</sub> levels (0, 5, 10, 15 and 20% CO<sub>2</sub>). All gas exchange data were expressed as mmol kg<sup>-1</sup> s<sup>-1</sup>.

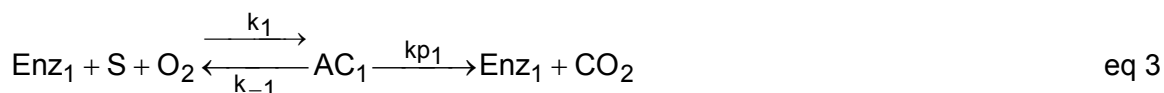
### Statistical analysis

The collected experimental data were analysed statistically with the iterative non-linear regression routine of the statistical package Genstat 5 (release 3.1, Lawes Agricultural Trust, Rothamsted Experimental Station, UK). For each of the products, the data of all O<sub>2</sub>-CO<sub>2</sub>-temperature combinations were analysed together, using the model formulation of Eqs. (1) and (10), together with the temperature dependence according to Arrhenius' law (Eq. (12)) applied to V<sub>mO<sub>2</sub></sub> and V<sub>mCO<sub>2</sub>(f)</sub>. The reference temperature for Arrhenius' law was fixed at 10°C, in the middle of the applied temperature range. The data were analysed for each product, using simultaneously O<sub>2</sub>, CO<sub>2</sub> and temperature as independent variables and CO<sub>2</sub> production and O<sub>2</sub> consumption as dependent variables (multi-response, multivariate, non-linear regression analysis). The nonlinear equations were applied directly, without transformation to data or equations.

## Model development

### Respiration

Consumption of O<sub>2</sub> by respiration as formulated by Eq. (1) is based on a combined type of inhibition. So both the enzyme-substrate complex and the enzyme itself are inhibited by CO<sub>2</sub>. Consumption of O<sub>2</sub> by respiration can be simplified and described as:



with S<sup>-</sup> substrate, Enz<sub>1</sub><sup>-</sup> a simplified representation of the enzyme system involved and AC<sub>1</sub><sup>-</sup> the intermediate enzyme-substrate complex. This complex is in steady state with its

constituents, O<sub>2</sub>, substrate and enzyme, and forms by a single reaction the product CO<sub>2</sub>. The steady state reaction is characterized by its forward and backward reaction rates  $k_1$  and  $k_{-1}$ . The reaction rate for the formation of the product CO<sub>2</sub> is given by  $kp_1$ . Competitive inhibition by CO<sub>2</sub> can be depicted as:



So both the inhibitor CO<sub>2</sub> and the reagent O<sub>2</sub> compete for the same active site of the enzyme. However, the complex formed with CO<sub>2</sub> (AC<sub>2</sub>) generates no further products. The steady state reaction of the competitive inhibition is characterized by its forward and backward reaction rates  $k_2$  and  $k_{-2}$ . Finally, uncompetitive inhibition can be presented as:



where CO<sub>2</sub> removes complex AC<sub>1</sub> from the active pool by formation of complex AC<sub>3</sub>. The steady state reaction of the uncompetitive inhibition is characterized by its forward and backward reaction rates  $k_3$  and  $k_{-3}$ .

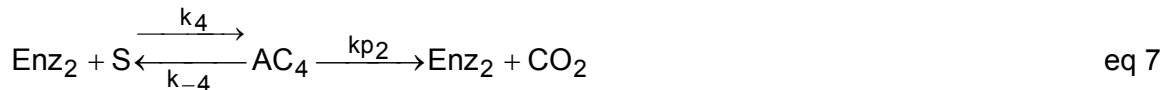
Eq. (1) is derived from the reaction schemes Eqs. (3)–(5). As usual with derivations of Michaelis–Menten type kinetics, steady state approximation is applied. Furthermore, the assumption is made that the substrate for respiration is abundantly available and hence can assumed to be relatively constant. The Michaelis constants from Eq. (1) are all based on the individual rate constants from the reaction Eqs. (3)–(5) and are defined by:

$$\begin{aligned} V_{m_{O_2}} &= kp_1 \cdot [\text{Enz}_{1,0}] \\ K_{m_{O_2}} &= \frac{k_{-1} + kp_1}{k_1 \cdot [S_0]} \\ K_{mc_{CO_2}} &= \frac{k_{-2}}{k_2} \\ K_{mi_{CO_2}} &= \frac{k_{-3}}{k_3} \end{aligned} \quad \text{eq 6}$$

where  $[\text{Enz}_{1,0}]$ =the concentration of enzyme initially available and  $[S_0]$ =the concentration of substrate initially available.

### **Fermentation**

The production of CO<sub>2</sub> by fermentation as formulated in the second term of Eq. (2) is based on a competitive inhibition by O<sub>2</sub>. However, the current data on fermentation also show some inhibition by CO<sub>2</sub>. Therefore, the model is extended with another competitive inhibition, but now by CO<sub>2</sub>. The production of CO<sub>2</sub> by fermentation can be simplified and described as:



with S=substrate, Enz<sub>2</sub>=a simplified representation of the enzyme system involved and AC<sub>4</sub>=the intermediate enzyme–substrate complex. This complex is in steady state with its constituents, substrate and enzyme, and forms by a single reaction the product CO<sub>2</sub>. The steady state reaction is characterized by its forward and backward reaction rates  $k_4$  and  $k_{-4}$ . The reaction rate for the formation of the product CO<sub>2</sub> is given by  $kp_2$ . Competitive inhibition by O<sub>2</sub> can be depicted as:



where  $O_2$  removes enzyme from the active pool by formation of complex  $AC_5$ . The steady state reaction of the competitive inhibition is characterized by its forward and backward reaction rates  $k_5$  and  $k_{-5}$ . Finally, the competitive inhibition by  $CO_2$  can be presented as:



where  $CO_2$  removes enzyme from the active pool by formation of complex  $AC_6$ . The steady state reaction of the uncompetitive inhibition is characterized by its forward and backward reaction rates  $k_6$  and  $k_{-6}$ .

The fermentative part of  $CO_2$  production ( $V_{CO_2(f)}$ ) can be derived from the reaction schemes Eqs. (7)–(9), resulting in a description of the total  $CO_2$  production ( $V_{CO_2}$ ) by:

$$V_{CO_2(f)} = \frac{Vm_{CO_2(f)}}{\left(1 + \frac{[O_2]}{Kmc_{O_2(f)}} + \frac{[CO_2]}{Kmc_{CO_2(f)}}\right) \cdot Km_{CO_2(f)} + 1} \quad \text{eq. 10}$$

$$V_{CO_2} = RQ_{ox} \cdot V_{O_2} + V_{CO_2(f)}$$

with  $Km_{CO_2(f)}$  the Michaelis constant for fermentative  $CO_2$  production (%), and  $Kmc_{CO_2(f)}$  the Michaelis constant for the competitive inhibition of fermentation by  $CO_2$  (%). The Michaelis constants are again all based on the individual rate constants as presented in the preceding reactions: Eqs. (7)–(9). They are defined as:

$$Vm_{CO_2(f)} = kp_2 \cdot [Enz_{2,0}]$$

$$Km_{CO_2(f)} = \frac{k_{-4} + kp_2}{k_4 \cdot [S_0]}$$

$$Kmc_{O_2(f)} = \frac{k_{-5}}{k_5} \quad \text{eq 11}$$

$$Kmc_{CO_2(f)} = \frac{k_{-6}}{k_6}$$

where  $[Enz_{2,0}]$  = the concentration of enzyme initially available and  $[S_0]$  = the concentration of substrate initially available. Again, steady state approximation is applied and the substrate for fermentation is assumed to be abundantly available and hence is assumed to be constant. Because of the limited information available on fermentation, the four parameters from Eq. (11) can not be estimated together. The formulation of the fermentative  $CO_2$  production is overparameterised by the presence of the parameter  $Km_{CO_2(f)}$ . This parameter has therefore been fixed at a relative value of 1%. This approach differs from the one applied by Peppelenbos et al. (1998) who resolved it by removing the parameter  $Km_{CO_2(f)}$ . As a consequence, their remaining three parameters are no longer defined according to Michaelis–Menten (as in Eq. (11)) but all include  $k_4$ ,  $k_4$ ,  $kp_2$  and  $[S_0]$ .

### Temperature dependence

Eqs. (6) and (11) show that  $Vm_i$  is directly proportional to a single rate constant while each  $Km_i$  is a ratio of rate constants. Each of the mentioned reaction rates depends on temperature, presumably according to Arrhenius' law:

$$k_i = k_{i,ref} \cdot e^{\frac{Ea_i}{R_{gas}} \left( \frac{1}{T_{ref}} - \frac{1}{T} \right)} \quad \text{eq 12}$$

$R_{gas}$  = gas constant ( $8.314 \text{ J mol}^{-1} \text{ K}^{-1}$ ). The parameter  $k_{i,ref}$  stands for the reaction rate constant at the arbitrarily chosen reference temperature  $T_{ref}$  (K). The energy of activation  $Ea_i$  expresses the dependence of the reaction rate  $k_i$  on temperature  $T$  (K). As the Michaelis constants  $Vm_{O_2}$

and  $V_{m_{CO_2(f)}}$  are directly related to  $kp_1$  and  $kp_2$  respectively, they would also depend on temperature according to Arrhenius' law. Assuming that the rate constants of the steady state reactions have activation energies of roughly the same magnitude, each  $Km_i$ , being a ratio of rate constants, would be expected to be relatively independent of temperature, as the difference in activation energies is most probably much smaller than the individual activation energies (Eq. (13)).

$$\frac{k_i}{k_j} = \frac{k_{i,ref} \cdot e^{\frac{E_{a_i}}{R_{gas}} \left( \frac{1}{T_{ref}} - \frac{1}{T} \right)}}{k_{j,ref} \cdot e^{\frac{E_{a_j}}{R_{gas}} \left( \frac{1}{T_{ref}} - \frac{1}{T} \right)}} = \frac{k_{i,ref}}{k_{j,ref}} \cdot e^{\frac{E_{a_i} - E_{a_j}}{R_{gas}} \left( \frac{1}{T_{ref}} - \frac{1}{T} \right)}$$

eq 13

with  $E_{a_i} = E_{a_j} \Rightarrow \frac{k_{i,ref}}{k_{j,ref}}$

Preliminary analysis of the current data on gas exchange, allowing a temperature dependence for both the  $V_{m_i}$  as for  $Km_i$ , did not reveal a significant temperature dependence for  $Km_i$ . Only  $V_{m_i}$  exhibited a significant temperature dependence. This supports the assumptions made. From the preceding, it is concluded that the parameters  $V_{m_{O_2}}$  and

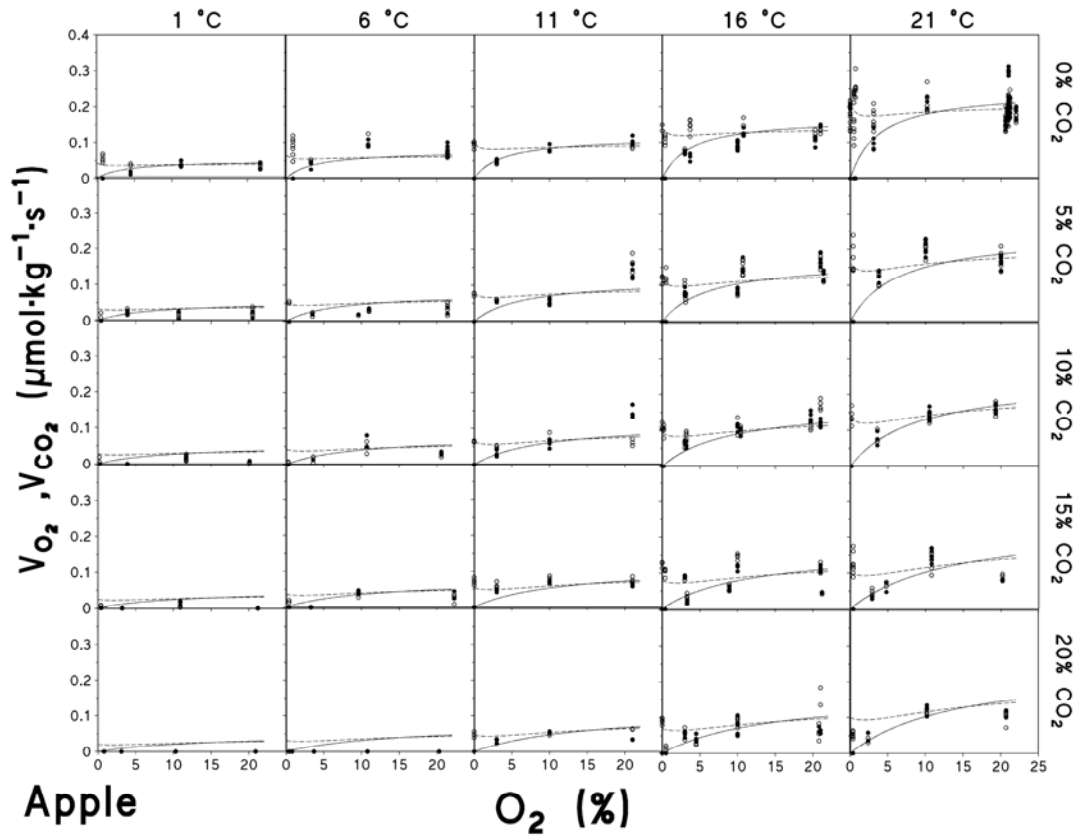


Figure 8-1  $O_2$  consumption ( $V_{O_2}$  in  $mmol\ kg^{-1}\ s^{-1}$ ; closed symbols and continuous lines) and  $CO_2$  production ( $V_{CO_2}$  in  $mmol\ kg^{-1}\ s^{-1}$ ; open symbols and dotted lines) of 'Golden Delicious' apple as a function of  $O_2$  (x-axes),  $CO_2$  (rows of graphs) and temperature (columns of graphs). The symbols are measured values while the lines are simulated values.

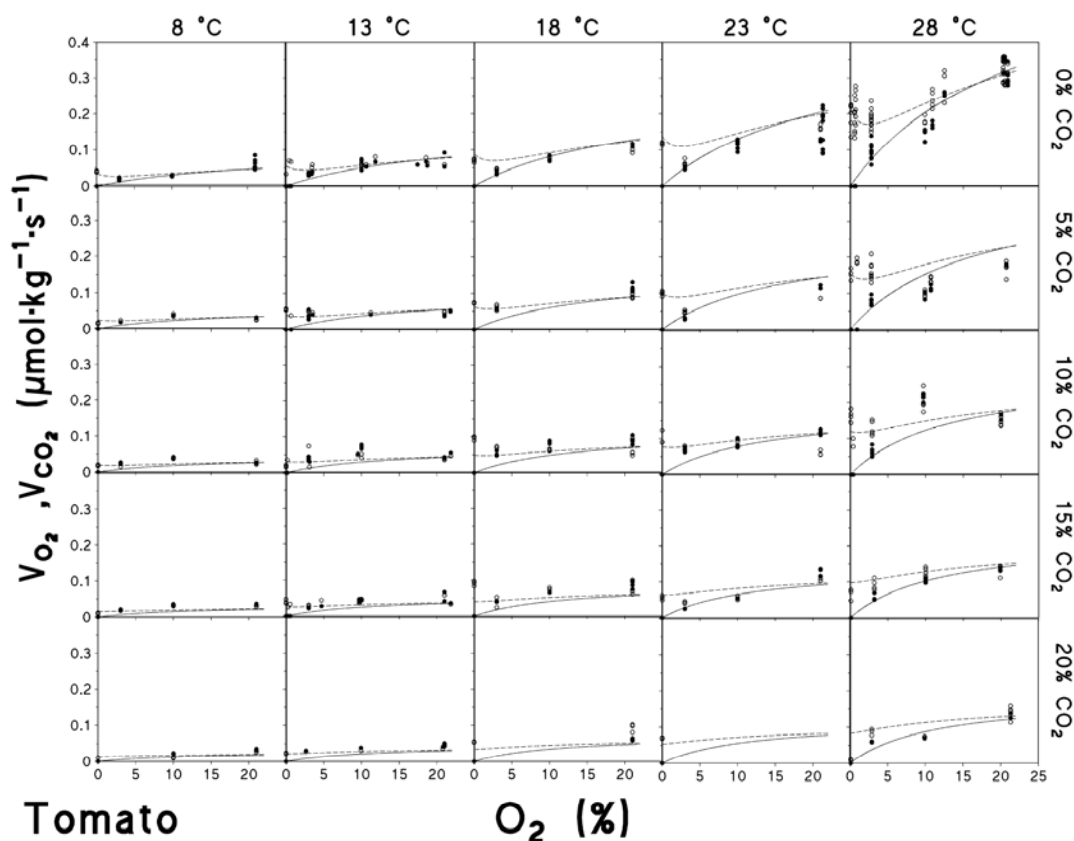


Figure 8-2  $O_2$  consumption ( $V_{O_2}$  in  $\text{mmol kg}^{-1} \text{ s}^{-1}$ ; closed symbols and continuous lines) and  $CO_2$  production ( $V_{CO_2}$  in  $\text{mmol kg}^{-1} \text{ s}^{-1}$ ; open symbols and dotted lines) of tomato as a function of  $O_2$  (x-axes),  $CO_2$  (rows of graphs) and temperature (columns of graphs). The symbols are measured values while the lines are simulated values.

$V_{m_{CO_2(f)}}$  will most strongly depend on temperature according to Arrhenius' law whereas each of the  $Km_i$  will be relatively temperature independent and can be treated as constants.

## Experimental results

The data collected for apple ('Golden Delicious') are shown in Figure 8-1 as symbols. In spite of a distinct source of variation from the use of different batches of products over the years and from the different measuring techniques applied by the different research partners, the effects of gas conditions and temperature on gas exchange are clearly visible. For apple (Figure 8-1), there was a clear effect of  $O_2$  concentration on the level of respiration and on the occurrence of fermentation. Respiration increased with increasing  $O_2$  concentration while fermentation increased when  $O_2$  concentration decreased. Both the maximum observed respiration at high oxygen and the maximum fermentation at 0%  $O_2$  depended on temperature (Figure 8-1).  $CO_2$  inhibited both respiration and fermentation. The overall effect of temperature was more pronounced than the effect of  $CO_2$  (Figure 8-1). Data for tomato are shown in Figure 8-2. The applied temperatures were different from those applied to apple and chicory (Figure 8-3) because of the susceptibility of tomato to low temperatures. The behaviour of respiration and fermentation of tomato, as a function of  $O_2$ ,  $CO_2$  and temperature, was in principle comparable to that of apple except that tomato expressed this behaviour at a higher temperature level. In contrast to apple and tomato, there was no effect of  $CO_2$  on respiration or fermentation for chicory (Figure 8-3). The respiration of chicory responded more strongly to an increase in  $O_2$  at low oxygen conditions than did that of apple and tomato. Furthermore, chicory exhibited the highest rate of respiration.



## Model results

The results of the multi-response, multi-variate, non-linear regression analysis of each of the products are presented in Table 8-2. During the iterative process of non-linear regression analysis on the data for apple and chicory, some of the parameters  $Km_i$  tended towards extreme large values. This indicates that the type of inhibition was statistically not relevant.

For these cases,  $Km_i$  was fixed at  $+\infty$  in the final analysis. The model is capable of explaining 80–90% of the observed variance ( $R^2_{adj}$ ); this in spite of the distinct sources of variation present. The accuracy of estimation of the separate parameters is given by their standard errors (%). The simulated data for apple, tomato and chicory generated by the model, applying the estimated parameters from Table 8-2, are shown as solid lines in Figure 8-1 - Figure 8-3, respectively.

Table 8-2 Results of the non-linear regression analysis using  $O_2$ ,  $CO_2$ , and temperature simultaneously as independent variables

Product	Parameter		
	Chicory	Tomato	Apple
Parameters describing respiration (estimate (S.E.)) <sup>a</sup>			
$Vm_{O_2,ref}$	0.106 (4.7)	0.112 (2.8)	0.122 (11)
$EaVm_{O_2}$	52 875 (4.1)	67 139 (2.2)	67 338 (2.8)
$Km_{O_2}$	3.76 (16)	2.70 (8.9)	23.2 (19)
$Kmc_{CO_2}$	7.36 (17)	$+\infty$ (-)	21.3 (33)
$Kmu_{CO_2}$	$-\infty$ (-) <sup>c</sup>	$-\infty$ (-)	7.85 (23)
$RQ_{ox}$	0.90 (2.5)	0.84 (3.2)	0.91 (2.8)
Parameters describing fermentation (estimate (S.E.)) <sup>b</sup>			
$Vm_{CO_2(f),ref}$	0.178 (6.6)	0.130 (6.5)	0.0817 (8.3)
$EaVm_{CO_2(f)}$	52 358 (7.8)	71 588 (6.5)	65 159 (6.1)
$Km_{CO_2(f)}$	1(-) <sup>c</sup>	1(-) <sup>c</sup>	1(-) <sup>c</sup>
$Kmc_{O_2(f)}$	1.01 (17)	0.541 (23)	1.37 (18)
$Kmc_{CO_2(f)}$	9.63 (14)	$+\infty$ (-) <sup>c</sup>	6.49 (14)
$R^2_{adj}$ (%)	83.0	86.8	87.0
$n^d$	995	917	909
<p>The standard errors (S.E.) are expressed as a percentage, relative to the estimated value.</p> <p><sup>a</sup> <math>Vm_{O_2,ref}</math>=the maximum <math>O_2</math> consumption rate (<math>mmol\ kg^{-1}\ s^{-1}</math>) at reference temperature <math>T_{ref}</math> (<math>=10^\circ C</math>); <math>EaVm_{O_2}</math>=energy of activation (<math>J\ mol^{-1}</math>) of rate constant <math>Vm_{O_2}</math>; <math>Km_{O_2}</math>=Michaelis constant for <math>O_2</math> consumption (%); <math>Kmi_{CO_2}</math>=Michaelis constant for inhibition of <math>O_2</math> consumption by <math>CO_2</math> (%); <math>l=c</math>: competitive; <math>l=u</math>: uncompetitive; <math>RQ_{ox}</math>=respiration quotient.</p> <p><sup>b</sup> <math>Vm_{CO_2(f),ref}</math>=the maximum <math>CO_2</math> production rate (<math>mmol\ kg^{-1}\ s^{-1}</math>) at reference temperature <math>T_{ref}</math> (<math>=10^\circ C</math>); <math>EaVm_{CO_2(f)}</math>=energy of activation (<math>J\ mol^{-1}</math>) of rate constant <math>Vm_{CO_2}</math>; <math>Km_{CO_2(f)}</math> the Michaelis constant for fermentative <math>CO_2</math> production (%); <math>Kmc_{i(f)}</math>=Michaelis constant for competitive inhibition of fermentative <math>CO_2</math> production by either <math>O_2</math> or <math>CO_2</math> (%).</p> <p><sup>c</sup> <math>R^2_{adj}</math>=percentage variance accounted for.</p> <p><sup>d</sup> <math>n</math>=number of data points.</p> <p><sup>e</sup> Fixed value, so no standard error.</p>			

## Discussion

Although each product exhibited its own gas exchange pattern as a function of  $O_2$ ,  $CO_2$  and temperature, they are all well described by the same model. This proves the generic features of the developed model. The quantification of interactive effects of  $O_2$ ,  $CO_2$  and temperature is quite difficult when interpreting the results by sight. However, analyses with the developed model do enable discrimination between the effects of the factors  $O_2$ ,  $CO_2$  and temperature. These effects will be discussed in the following sections.

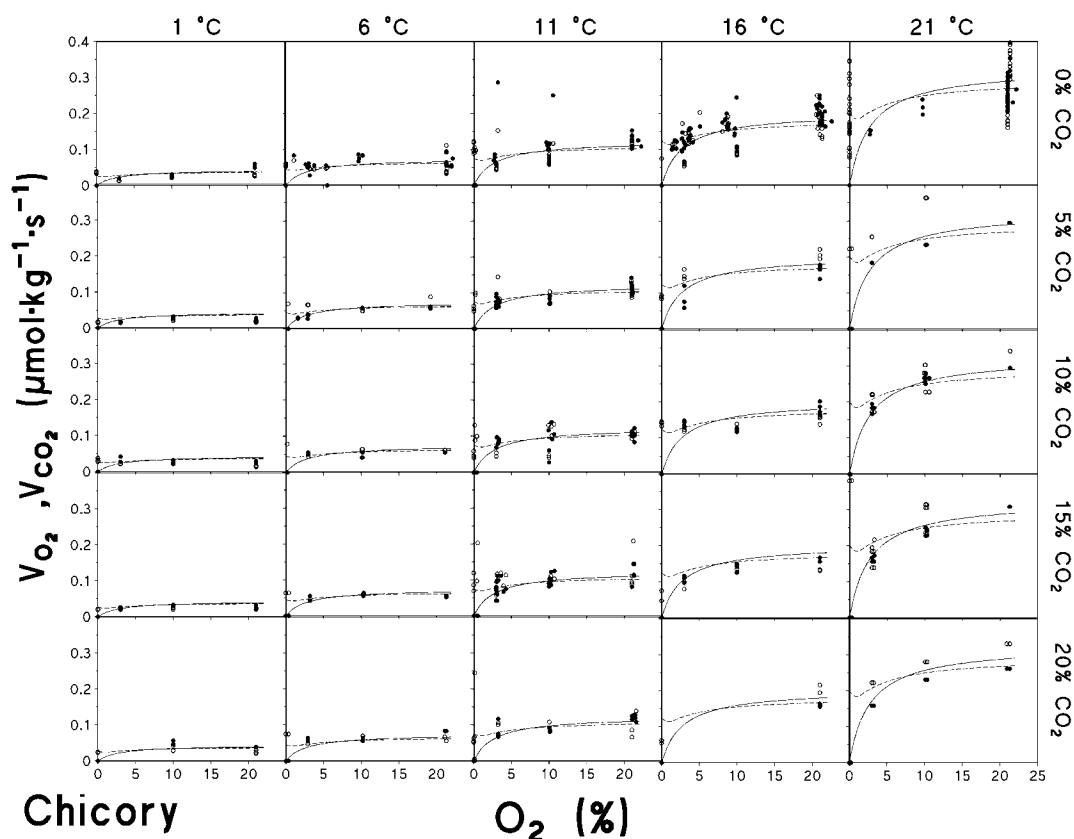


Figure 8-3  $O_2$  consumption ( $V_{O_2}$  in  $\text{mmol kg}^{-1} \text{ s}^{-1}$ ; closed symbols and continuous lines) and  $CO_2$  production ( $V_{CO_2}$  in  $\text{mmol kg}^{-1} \text{ s}^{-1}$ ; open symbols and dotted lines) of chicory as a function of  $O_2$  (x-axes),  $CO_2$  (rows of graphs) and temperature (columns of graphs). The symbols are measured values while the lines are simulated values.

### ***Interpretation of the model parameters and their values***

#### ***Effect of temperature on respiration***

The maximum  $O_2$  consumption rate at a reference temperature of  $10^\circ\text{C}$  ( $V_{mO_2,ref}$ ) was comparable for the three products studied. Apple, however, reacted less to temperature than tomato and chicory (Figure 8-1 as compared to Figure 8-2 and Figure 8-3), which is expressed by its lower energy of activation ( $E_{aV_{mO_2}}$ ; Table 8-2).

#### ***Effect of $O_2$ on respiration***

The parameter  $Km_{O_2}$  is a measure for the saturation of respiration with oxygen. It represents the oxygen concentration at which half the maximum respiration rate ( $1/2 V_{mO_2}$ ) is reached, assuming no inhibition by  $CO_2$ . Chicory and apple have a comparable  $Km_{O_2}$  (respectively 2.70 and 3.76%) while tomato has a much higher  $Km_{O_2}$  of 23.2%. Thus tomato shows a less steep incline of respiration as a function of  $O_2$  as can be seen in Figure 8-2. This is probably due to the relatively impermeable skin of tomato (De Vries et al., 1996). As a consequence the internal oxygen concentration will not increase proportionally to the external concentrations applied.

#### ***Inhibition of respiration by $CO_2$***

The value for  $Kmi_{CO_2}$  is a measure of the extent to which respiration can be inhibited by  $CO_2$  (either competitive or uncompetitive). A high value for  $Kmi_{CO_2}$  implies that the backward reaction of the inhibition is much faster than the forward reaction and hence the inhibition by  $CO_2$  is not occurring. The consequences in terms of the mathematical formulation are

outlined in Table 8-1. Apple only showed the competitive inhibition by CO<sub>2</sub> (Table 8-2). Chicory showed no inhibition at all (both  $Km_{CO_2}$ , and  $Kmu_{CO_2}$  are set to  $+\infty$ ; Table 8-2). The respiration of tomatoes was prone to a slight competitive inhibition (as  $Kmu_{CO_2}$  is of the same magnitude as CO<sub>2</sub>, but still quite large; 21.3%) and a clear uncompetitive inhibition by CO<sub>2</sub> ( $Kmu_{CO_2} = 7.85\%$ ). The apparent nil respiration of apple at low temperature and high CO<sub>2</sub> was probably due to the limited sensitivity of the applied measuring technique relative to the high background CO<sub>2</sub>.

#### *Effect of O<sub>2</sub> on the inhibition of respiration by CO<sub>2</sub>*

The effect of competitive inhibition by CO<sub>2</sub> can be counteracted by raising the level of O<sub>2</sub>, as O<sub>2</sub> and CO<sub>2</sub> are competing for the same active sites. In the case of uncompetitive inhibition, as for tomato, the maximum respiration level  $V_{mO_2}$  can not be reached by raising O<sub>2</sub>, because there will always be a certain amount of intermediate complex (AC<sub>1</sub>) available for CO<sub>2</sub> to react with. This can also be deduced from Eq. (1), as in the case of competitive inhibition:

$$\lim_{[O_2] \rightarrow \infty} (V_{O_2}) = V_{mO_2}$$

but in the case of uncompetitive inhibition:

$$\lim_{[O_2] \rightarrow \infty} (V_{O_2}) = \frac{V_{mO_2}}{1 + \frac{[CO_2]}{kmu_{CO_2}}}$$

So, in the case of uncompetitive inhibition and as long as CO<sub>2</sub> is present, the rate of respiration will always be below the maximum rate,  $V_{mO_2}$ .

#### *Respiration quotient*

The estimated respiration quotient for oxidative metabolism ( $RQ_{ox}$ ) is very similar for the products studied (Table 8-2). For each product it is less than unity: the O<sub>2</sub> consumption was always higher than the oxidative CO<sub>2</sub> production.

#### *Effect of temperature on fermentation*

The maximum rate of fermentative CO<sub>2</sub> production at  $T_{ref}$ , ( $V_{mCO_2(f),ref}$ ) was highest for apple (0.178 mmol kg<sup>-1</sup> s<sup>-1</sup>) and lowest for tomato (0.0817 mmol kg<sup>-1</sup> s<sup>-1</sup>). The accompanying energy of activation ( $Ea_{V_{mCO_2(f)}}$ ) was least for apple while for chicory and tomato this value was comparable (Table 8-2).

#### *Inhibition of fermentation by O<sub>2</sub>*

The value for  $Km_{CO_2(f)}$  is a measure of the extent to which fermentation can be competitively inhibited by O<sub>2</sub>. A high value for  $Km_{CO_2(f)}$  implies that inhibition will not take place. Chicory has the lowest value for  $Km_{CO_2(f)}$  (Table 8-2). Thus, fermentation is already inhibited at relative low O<sub>2</sub> levels. Tomato, with the highest value for  $Km_{CO_2(f)}$ , shows fermentative CO<sub>2</sub> production at relatively high levels of O<sub>2</sub>. This is probably again due to the relatively impermeable skin of tomato (De Vries et al., 1996), resulting in internally hypoxic conditions at O<sub>2</sub> concentrations well above zero.

#### *Inhibition of fermentation by CO<sub>2</sub>*

The value for  $Km_{CO_2(f)}$  is a measure of the extent to which fermentation can be competitively inhibited by CO<sub>2</sub>. A high value for  $Km_{CO_2(f)}$  implies that the inhibition is not effective. Chicory did not exhibit inhibition of fermentation by CO<sub>2</sub>, whereas both apple and tomato did. This can clearly be seen in the increasing CO<sub>2</sub> levels in the Figure 8-1 and Figure 8-2 in the plots at the same temperature at 0% O<sub>2</sub>.

#### **Accuracy of parameter estimates**

The accuracy of the parameter estimates is reflected in the standard errors of the estimates. A parameter with a standard error of up to about 10% of the estimated value can be regarded as accurate. Parameters with standard errors up to about 50% are still significantly different from zero but are less defined with increasing standard error. The parameters  $V_{m_i}$ ,  $Ea_i$ , and  $RQ_{ox}$  have for each of the three products studied, small standard errors and can be

considered as well defined. The parameters  $Kmc_{O_2(f)}$  and  $Kmc_{CO_2(f)}$  consistently had somewhat larger standard errors (14–23%) due to the limited amount of information available in the data on the inhibition of fermentation by either  $O_2$  or  $CO_2$ . For tomatoes,  $Kmc_{CO_2(f)}$  and  $Kmu_{CO_2(f)}$  have somewhat larger standard errors because of the coexistence of both competitive and uncompetitive inhibition of respiration by  $CO_2$ . The discrimination between the two types of inhibition is statistically not obvious. The remaining parameters still have acceptable standard errors and are significantly different from zero.

### **Values from the literature**

In this research  $Vm_i$  appeared to be a function of temperature according to Arrhenius' law. When comparing parameter estimates with values from the literature, it has to be taken into account that the published values of  $Vm_{O_2}$  (and  $Vm_{CO_2(f)}$ ) are only valid for the specified experimental temperature. To compare the current results with those from the literature, first the value of  $Vm_{O_2}$  for a specific temperature has to be calculated according to Arrhenius' law (Eq. (12)) with the estimates of  $Vm_{O_2,ref}$  and  $Ea_{Vm_{O_2}}$  (Table 8-2). When comparing the parameter estimates for apple with published data on 'Golden Delicious' apples from Peppelenbos and Van't Leven (1996), only slightly different values are found. They found a value for  $Vm_{O_2}$  at 19°C of 23 ml kg<sup>-1</sup> h<sup>-1</sup> (0.286 mmol kg<sup>-1</sup> s<sup>-1</sup>) and a  $Km_{O_2}$  of 6.17% as compared to a  $Vm_{O_2}$  at 19°C of 0.212 mmol kg<sup>-1</sup> s<sup>-1</sup> and a  $Km_{O_2}$  of 3.76% as estimated for the current set of data. Peppelenbos and Van't Leven (1996) found only a slight competitive inhibition by  $CO_2$  ( $Kmc_{CO_2} = 47.8\%$ ) as compared to the current  $Kmc_{CO_2}$  of 7.36%. This difference can be explained by the fact that they only made measurements at 0 and 5%  $CO_2$  while the current data are gathered over a range of 0–20%  $CO_2$ . In the range from 0 to 5%  $CO_2$  the inhibitive effect is indeed very small (Figure 8-2; 21°C: 0%  $CO_2$  as compared to 21°C: 5%  $CO_2$ ).

The parameter estimates on cut chicory at 8°C from Peppelenbos and Van't Leven (1996) are clearly different from the current estimates on intact chicory. The  $Vm_{O_2}$  Peppelenbos and Van't Leven (1996) found (2 mmol kg<sup>-1</sup> h<sup>-1</sup>:0.56 mmol kg<sup>-1</sup> s<sup>-1</sup>) is six times the current value of 0.092 mmol kg<sup>-1</sup> s<sup>-1</sup> (at 8°C). This can be attributed to the incurred stress and damage by the cutting. Furthermore, the relatively inactive part of chicory (the basal part) was removed and only the active leaf tissue was used for gas exchange measurements (Peppelenbos and Van't Leven, 1996). The  $Km_{O_2}$  is the same for cut (2.61%) as for intact chicory (2.70%). Cut chicory showed a clear competitive inhibition ( $Kmc_{CO_2} = 3.41\%$ ) while intact chicory showed no inhibition of respiration by  $CO_2$  ( $Kmc_{CO_2}$  set to  $+\infty$ ). Apparently, the basal metabolism can not be inhibited by  $CO_2$ , while the induced wound respiration can be inhibited by  $CO_2$ .

For tomato, Peppelenbos and Van't Leven (1996) reanalysed data from Yang and Chinnan (1988). The value they found for  $Vm_{O_2}$  at 21°C (22.4 ml kg<sup>-1</sup> h<sup>-1</sup>:0.278 mmol kg<sup>-1</sup> s<sup>-1</sup>) is comparable to a  $Vm_{O_2} = 0.356$  mmol kg<sup>-1</sup> s<sup>-1</sup> for the current data. Also the value for  $Km_{O_2}$  is comparable (Peppelenbos and Van't Leven (1996): 17.1%; current research: 23.2%). Peppelenbos and Van't Leven (1996) attributed all of the inhibitory effect of  $CO_2$  on respiration to the uncompetitive inhibition ( $Kmu_{CO_2} = 15.5\%$ ). The current data exhibit a somewhat larger uncompetitive  $CO_2$  effect ( $Kmu_{CO_2} = 7.85\%$ ) and also a slight competitive inhibition ( $Kmc_{CO_2} = 21.3\%$ ).

### **Temperature dependence of Michaelis–Menten parameters**

The line of reasoning applied to deduce which of the parameters ( $Vm_i$  or  $Km_i$ ) should be expected to depend on temperature, was also applied by Kovárová et al. (1996) for the temperature dependence of bacterial growth. They observed a constant value for the  $Km$  of bacterial growth while the maximum growth rate depended on temperature.

This approach is also confirmed by other experimental data from the literature on respiration. Ratti et al. (1996), who measured respiration of cauliflower as a function of  $O_2$ ,  $CO_2$  and temperature, analysed their data using the formulation of uncompetitive inhibition. They applied a temperature dependence to both  $Km_{O_2}$  and  $Vm_{O_2}$ . After reanalysing their data, it appeared that the energy of activation for  $Km_{O_2}$  ( $Ea_{Km_{O_2}}$ ) is less than one third of the energy of activation for  $Vm_{O_2}$  ( $Ea_{Vm_{O_2}}$ ). But, what is more important, the standard error of the estimate of  $Ea_{Km_{O_2}}$  is 177% while the standard error of the estimate of  $Ea_{Vm_{O_2}}$  is only 8%. This

implies that  $Ea_{Km_{O_2}}$  is in fact not significantly different from zero. As a consequence,  $Km_{O_2}$  can be regarded as temperature independent.

Song et al. (1992), applied the model of uncompetitive inhibition to data on blueberry stored at various temperatures. They also found values for  $Vm_{O_2}$  increasing with increasing temperatures, while  $Km_{CO_2}$  and  $Kmuc_{CO_2}$  exhibited quite erratic behaviour as a function of temperature but remained relatively constant. These values, however, resulted from analyses for each temperature separately. After generating data using their model, the generated data were reanalysed applying the current integrated approach. This resulted in assigning the complete temperature dependence to  $Vm_{O_2}$ , treating  $Km_{O_2}$  and  $Kmuc_{CO_2}$  as constants, with an explained part of 96%. This shows again that the approach of attributing temperature dependence completely to  $Vm_{O_2}$  (and  $Vm_{CO_2(f)}$ ) is correct.

Cameron et al. (1994), also studied  $O_2$  consumption of blueberry assuming no inhibition by  $CO_2$ . For this purpose, they used sealed packages with product. They found a clear temperature effect on both  $Vm_{O_2}$  and  $Km_{O_2}$  which could be confirmed by reanalysing their data with the current approach. However, their assumption on a lack of inhibition by  $CO_2$  is in sharp contrast with the results of Song et al. (1992). If the assumption of Cameron et al. (1994) was not valid, meaning that in fact their blueberries were liable to  $CO_2$  inhibition, the temperature effect they observed for  $Km_{O_2}$  is probably the result of temperature on fermentation. Fermentation is responsible for the accumulation of  $CO_2$  which, in turn, can inhibit respiration of blueberry (Song et al., 1992). In this case, the effect of temperature on  $Vm_{CO_2(f)}$  is completely incorporated in the observed apparent temperature effect on  $Km_{O_2}$ .

## Conclusion

To improve the application of the model, gas exchange was formulated as a function of environmental gas concentrations. Gas diffusion from the atmosphere into the product is implicitly incorporated in the proposed mechanism and the estimated parameter values. Each of the steps of the mechanism described can be considered as a chain of physiological and biochemical events. The fundamental, physiologically based gas exchange model presented, has the advantage of interpreting the model parameters in their physiological context. As a result of the integrated approach, the effect of temperature could completely be attributed to  $Vm_{O_2}$  and  $Vm_{CO_2(f)}$ , treating the other parameters as constants. The parameter estimates seemed to be of general value as they could be derived using a compilation of different batches used in this research and appeared to be largely comparable to prior estimates from the literature. The applied model is suitable for simulating gas exchange under various, constant or dynamic, conditions. Based on this gas exchange model a tool was developed to simulate the complete system of modified atmosphere packages throughout a logistic chain under dynamic changing temperatures (Hertog et al., 1997a,b; Hertog and Tijskens, 1998).

## Acknowledgements

The work was financially supported by the European Community (EC-AIR project no. AIR2-CT-1326). The authors wish to thank the participants of the EC-AIR MASTER project: Institute Nationale de la Recherche Agronomique (INRA, Montfavet, France; data on chicory and tomato), Istituto di Industrie Agrarie (University of Pisa, Italy; data on apple), and Verbond van Belgische Tuinbouwveilingen (VBT, Leuven, Belgium; data on apple, chicory and tomato) for gathering the experimental data on respiration.

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**9**

**Photosystem 2 quantum yield as a measure of radical scavengers in chilling injury in cucumber fruits and bell peppers. A static, dynamic and statistical model.**

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Published in *Planta*, 1994, **194**, 478-486

## Abstract.

Cucumber fruits (*Cucumis sativus* L. cv Jessica) and green bell peppers (*Capsicum annuum* L. cv's Lokas and Medeo) were stored at different temperatures ranging from 2 to 12 °C. After three different storage periods, fruits from each temperature were transferred to 20 °C for 7 days to allow for the development of visual symptoms of chilling injury (CI). During storage, the photochemical quantum yield of photosystem 2 (PS II) in peel tissue adapted to darkness, was calculated from measurements of pulse amplitude modulated chlorophyll fluorescence. The decrease in PS II quantum yield during storage at low temperatures in darkness can be described as a temperature dependent inhibition of an enzyme according to Arrhenius assuming a negative activation energy. By comparison with the radical scavenger measurements of Hariyadi and Parkin (1991) it is postulated that the time and temperature dependence of the quantum yield parallels the diminution of radical scavenging activity at lower temperatures in cucumber and capsicum fruits. This is combined with an equation for the process of radical scavenging itself and an equation for the auto-catalytic radical producing lipid peroxidation reaction. These three basic processes lead to both a static and a dynamic model for the occurrence of chilling injury in low temperature sensitive plant tissue. A statistical fit of the measured data using the static model leads to the estimates of the different activation energies and reaction rates with a high degree of accuracy. The estimated values are in accord with what one would expect on the basis of knowledge of the processes leading to chilling injury and directly pointing to meaningful physico-chemical parameters.

**Keywords:** Chiling injury, mathematical model, radical scavenging, chlorophyll fluorescence.

## Introduction

Membrane degradation is a common response of plant tissue to different environmental stresses (Elstner and Konze 1976; Senaratna and McKersie 1986; Chrominski et al. 1986; Parkin and Kuo 1989). The plastid membranes have been shown to be very sensitive to chilling induced degradation in cucumber fruits (Kuo and Parkin 1989; Hariyadi and Parkin 1991) and in the pericarp tissue of tomato fruits (Nguyen and Mazliak 1990). Photoperoxidative processes have been shown to play a role in the development of chilling injury in light (Wise and Naylor 1987a, b; Asada and Takahashi 1987). The same processes have also been shown to be a major component leading to chilling induced lipid degradation in cucumber fruits stored in darkness (Hariyadi and Parkin 1991), although other effects have been shown to occur before the lipid peroxidation causes the actual decrease in glyco-lipids (Saczynska et al. 1993). Chilling of cucumber leaves at 5 °C in moderate light for 5 hours resulted in an uncoupling of chloroplast electron transport due to reversible release of the ATPase coupling factor CF<sub>1</sub> (Terashima et al. 1991a, b). Hence lipid peroxidation, although not necessarily the cause, has been shown to be a major component transducing chilling injury in chilling sensitive plant tissue. The direct cause lies more probably in low temperature-induced irreversible inhibition of enzymes (Graham and Patterson 1982) and a redistribution of cellular calcium (Minorsky 1985) probably leading to auto-formation of free radicals. The degree of oxidative stress experienced by the cell will be a function of the activity of free radical generating reactions on the one hand, and the activity of the free radical scavenging system on the other. Oxidative stress will result in lipid peroxidation (Halliwell 1987; Foyer et al., 1990) eventually leading to the break-down of membranes. Concomitantly a breakdown of PS II caused by oxygen radicals in the dark is to be expected (Gounaris and Selkirk 1992).

The thylakoid membranes of chloroplasts have been shown to be very sensitive to oxidative stress, as is the quantum yield of photosystem II (Sommersalo and Krause 1989; Schöner and Krause 1990) measured following dark adaptation to relax non-photochemical quenching. Consequently the quantum yield of dark adapted PS II was used as an indication

of stress in fruits. In previous research it was found that the quantum yield of PS II decreased when cucumbers were stored at low temperatures in darkness, a treatment that eventually led to the visual symptoms of chilling injury (Van Kooten et al. 1992). In the present experiments cold storage was carried out with cucumber fruits and green bell peppers.

The time and temperature dependency of the decrease in quantum yield of PS II could be treated mathematically as a decreasing enzyme activity. On the basis of this hypothesis a model was developed describing the cause and development of chilling injury. Although the model is a massive simplification of the cascade of events leading to the visual symptoms of chilling damage, it is still able to accurately describe and predict the time and temperature responses of cucumber fruits and bell peppers. The mathematical formulation also allows the combination of a large number of data measured with modulated chlorophyll fluorescence and visual assessments of chilling injury into one statistical analysis.

## Materials and methods

### ***Plant material.***

Cucumber (*Cucumis sativus* L. cv Jessica) and green bell peppers (*Capsicum annuum* L. cv's Lokas and Medeo) were purchased at a wholesale market. The cucumber fruits were fully mature and grown at one location in the months April-June. The bell peppers had reached full size but were still unripe. The fruits were uniform in size and appearance and free from any defects. Bell peppers which had started to change its colour were rejected. The fruits were randomly divided into 12 lots of 40 fruits each. The experiment was repeated one month later using fruits that had been grown in the period May-July. These fruits were from the same growers and of the same cultivars.

### ***Storage.***

The fruits were divided over 12 cold storage rooms where the temperature was set to 2, 4, 6, 8, 10 and 12 °C respectively (max. fluctuation 1 °C). The actual temperatures were recorded and averaged over the storage period. The relative humidity was kept between 80 and 90%. Each cell contained 40 cucumbers and 80 bell peppers, 40 of each cultivar. For every target temperature 2 cells were used. After 5, 12 and 19 days of storage 10 fruits of each cultivar were selected at random and moved from the cold storage cells to a storage cell at 20 °C and 70% relative humidity for 7 days to allow any chilling injury symptoms to develop. Fruits which were accidentally damaged during cold storage or started to change colour (bell peppers) were removed from the experiment. Hence, every combination of storage duration and temperature was repeated both in temperature and in time. Each data point represents an average value of 10 fruits.

### ***Chlorophyll fluorescence measurements.***

As the variable fluorescence yield following dark adaptation ( $F_v/F_m$ ) is a good measure of the quantum yield of oxygen evolution under limited irradiance (Björkman and Demmig 1987)  $F_v/F_m$  (nomenclature according to Van Kooten and Snel 1990) was measured with a Pulse Amplitude Modulated fluorometer (Walz, Effeltrich, Germany). The fruits were temporarily removed from the storage rooms and left to dark adapt under dim green light ( $<1 \mu\text{mol photons m}^{-2} \text{s}^{-1}$ ). A 3 mm transparent perspex spacer kept the surface of a selected fruit at a constant distance from the end of a light guide connected to the fluorometer. The intensity of the measuring beam was low enough that the fluorescence ( $F_0$ ) yield did not change after the fruit was placed under the fibre. Following the recording of the fluorescence yield, a saturating pulse ( $>5000 \text{ photons } \mu\text{mol m}^{-2} \text{s}^{-1}$  at the surface of the fruit) of 1 s duration was applied to the fruit and the frequency of the measuring light was increased 64-fold, a procedure which improves the signal to noise ratio of the measurement. During the pulse the maximum fluorescence yield was recorded ( $F_m$ ). This was done every day on 10 fruits of every cultivar randomly selected from each cold room. These two values were used to calculate  $F_v/F_m$ . The  $F_v/F_m$  was also measured immediately after the fruits were removed from the cold rooms to be stored at 20 °C and at the end of the 7 day period at that temperature.

**Visual assessment of chilling injury.**

After the fruits were retrieved from the cold room for storage at 20 °C they were assessed for chilling damage. The symptoms were pitting, depressions in the peel, incipient decay and tissue collapse with fungal infections as a secondary effect. If a fruit revealed any of these characteristics to any degree, it was categorized as chilling injured (CI). Thus a fraction was obtained of 10 fruits having acquired CI directly after cold storage periods of different durations and 7 days later at 20 °C.

**Description of the model****The basic chemical concept and mathematical formulation.**

As was stated in the introduction the two processes believed to be responsible for the onset of chilling injury are a redistribution of cellular calcium (Minorsky 1985) leading to free radical production combined with inhibition and/or denaturation of enzymes (Graham and Patterson 1982). As it was found that reduced glutathione and  $\alpha$ -tocopherol are the first components of the radical scavenging system in plastids to diminish upon chilling (Hariyadi and Parkin 1991), it is assumed that an enzyme (Z) leading to reduction of the glutathione, ascorbate and  $\alpha$ -tocopherol pools is inactivated by chilling ( $Z_{na}$ ).

The inhibition of the radical scavenging system is described as a time and temperature dependent process that has clear analogies with enzyme denaturation and bacterial growth inhibition. Yongsheng Feng et al. (1990) enhanced the scope of a system developed by Johnson et al. (1985) for describing responses of overall plant processes to temperature. As their model system is based only on the Boltzmann distribution of a component (e.g. enzyme) between an active and an inactive state, the nature of the system is inherently reversible. As chilling injury is an irreversible process, irreversible denaturation or deactivation has to be included. Eq. 1 describes the denaturation of the enzymes responsible for reducing the radical scavenging system in the plastids.

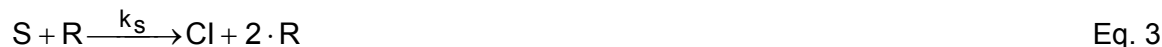
The enzyme Z is gradually converted at a low temperatures into an inactive enzyme  $Z_{na}$ .



The radical scavenging system itself is capable of neutralising free radicals (R) into inactive ones ( $R_{na}$ ):



Chilling injury is caused by the breakdown of membranes following the auto-catalytic attack of free radicals (R) on the unsaturated bonds (S) in the fatty acid side chains of lipids, a process known as lipid peroxidation (Halliwell 1987):



As we have no information about the actual flux of free radicals produced upon chilling, we assume a constant but very small influx of free radicals:



These four equations are the simplified chemical scheme describing the total cascade of events leading to chilling injury in chilling sensitive plant tissue.

Within an experiment at constant prestorage temperature, the results are quite straightforward. If, on the one hand, the total amount of radicals formed (Eq. 3 and Eq. 4) is less than the amount destroyed (Eq. 2), no radicals accumulate and no chilling injury symptoms will develop. On the other hand, if more free radicals are formed than can be scavenged, free radicals will accumulate and chilling injury symptoms will inevitably develop at some point in time. Neither situation allows for meaningful statistical analysis: in the first case no chilling injury will be observed, in the other case analysis of the chilling injury data can merely reveal information about the development of the symptoms, not about the

initiation. This can be described and analysed with a much more simple model (Eq. 3).

The real value of a complex model is for experiments combining different temperatures and times during cold storage and post-storage at other temperatures (usually higher). The results of these experiments are not straightforward. They are largely dependent on the relative influence of temperature on each of the described reactions (Eq. 1 to Eq. 4). This relative influence, expressed in the activation energies of the reaction rates, determines whether the remaining scavenging activity is sufficient to prevent development of chilling injury not only at low temperatures, but also at the subsequent higher temperatures. As a consequence, the activation energies of the four reactions will govern the complete process describing the complex behaviour of chilling injury at changing temperatures.

This qualitative chemical concept can be used as a basis for a quantitative model by deriving a set of four differential equations, assuming first order kinetics apply to each reaction:

$$-\frac{dS}{dt} = k_s \cdot S \cdot R \quad \text{Eq. 5}$$

$$\frac{dCl}{dt} = k_s \cdot S \cdot R \quad \text{Eq. 6}$$

$$\frac{dR}{dt} = k_s \cdot S \cdot R - k_r \cdot Z \cdot R + k_i \quad \text{Eq. 7}$$

$$-\frac{dZ}{dt} = k_d \cdot Z \quad \text{Eq. 8}$$

Though Michaelis-Menten kinetics or similar are usually encountered with enzyme catalyzed reactions, the specific conditions and simplifications tend to converge a cascade of the linear part of Michaelis-Menten kinetics into kinetics that adequately can be described by first order kinetics.

In this set of differential equations, each reaction rate ( $k_s$ ,  $k_r$ ,  $k_d$  and  $k_i$ ) is assumed to be temperature dependent according Arrhenius' law:

$$k = k_{ref} \cdot e^{\frac{E}{R_{gas}} \left( \frac{1}{T_{ref}} - \frac{1}{T_{abs}} \right)} \quad \text{Eq 9}$$

In Eq. 9  $k_{ref}$  stands for the reaction rate constant at the reference temperature ( $T_{ref}$ ).  $E$  represents the activation energy of the reaction and  $R_{gas}$  is the gas constant [ $8.314 \text{ J mole}^{-1} \text{ K}^{-1}$ ].  $T_{ref}$  and  $T_{abs}$  are the reference and the actual temperature respectively [K].

### ***The Pseudo static model:***

*Statistical analysis.* In order to be able to analyse statistically the measured data, an analytical solution of this set of differential equations at constant conditions is necessary. It should be emphasized that this analytic solution is necessary only for the statistical analysis and the generation of a pseudo static model of limited application. The analytical solution **only** applies to experiments at constant temperatures, and the pseudo static model **only** generates data for constant temperature situations.

To simplify the analytic integration, two assumption are made:

- (i) The amount of substrate S (double bonds in fatty acid chains) is present in abundance. This is a reasonable assumption considering the relative amounts of membranes (where the double bonds are situated) and free radicals in plant tissue. As a consequence S does not change during the reaction and Eq. 5 becomes zero.
- (ii) The continuous generation of radicals from reactions not covered within the model, is assumed to be zero (Eq. 4). Strictly speaking, this assumption is almost certainly invalid, but once under way the rate of auto-catalytic free radical formation is likely to greatly exceed free radical formation by other routes, even though these radicals may have

initially seeded the lipid peroxidation process.

The analytical solution of Eq. 8 can be generated directly, giving an expression for the amount of radical scavenger still active in the system:

$$Z = Z_0 \cdot e^{-k_d \cdot t} \quad \text{Eq. 10}$$

Combining Eq. 7 and Eq. 10, the expression for the increase or decrease of free radical concentration is obtained:

$$\frac{dR}{dt} = R \cdot (k_s \cdot S - k_z \cdot Z_0 \cdot (e^{-k_d \cdot t})) \quad \text{Eq. 11}$$

The analytical integral for this differential equation between 0 and time t is:

$$R = R_0 \cdot e^{k_s \cdot S \cdot t + k_r \cdot Z_0 \cdot \left( \frac{e^{-k_d \cdot t} - 1}{k_d} \right)} \quad \text{Eq. 12}$$

An equation for CI can be derived from Eq. 6 and Eq. 12, but this results in a rather cumbersome exponential form. Our means of scoring fruits for visually determined chilling injury imposes a limit on the value the model can reach as the score (CI) can only be as great as the total number of fruits whereas the derived equation (not shown) allows for a continually increasing limitless intensity for chilling injury. Therefore, an empirical logistic function, combining the expected exponential behaviour with a limiting value system, is used to relate the radical concentration with CI (the same type of formula can be derived from dynamic population statistics). The formulation of the equation has been slightly modified to suit this specific application:

$$CI = \frac{1}{1 + e^{(k_{CI} \cdot R + C_{CI})}} \quad \text{Eq. 13}$$

### ***The Dynamic model.***

The differential equations Eq. 6 to Eq. 8 are used directly in a dynamic model describing product behaviour in a changing sequence of events (temperature, time). This is the type of model most useful for describing and predicting the onset and development of chilling injury in products subjected to a changing environment as normally encountered in day to day practice. As already stated, the analytical solution, and the static and statistical model for constant environments, are, however important, only necessary for data analysis.

The language PERSONAL PROSIM (Sierenberg e.a. 1987, 1988) was used to realize the dynamic model described in general terms above. This language exploits a relatively new approach to modelling in which distinct parallel processes, that can be either continuous, discrete or mixed, can act as independent or unidirectionally or mutually dependent processes. Along with an object oriented type of programming, class component opportunities, including separate activation and deactivation of components, and a flexible and reliable integrating algorithm, this language offers an almost infinite number of possibilities.

The values for the model parameters are largely taken from the estimates obtained from the statistical analysis (see Results). Those parameters for which no estimates could be obtained (e.g.  $R_0$ ) were fixed to a probable value.

### ***Negative activation energies.***

Increasing deactivation of an enzyme or scavenger with decreasing temperature, implies a negative activation energy in the Arrhenius equation (Eq. 9). Although this is formally impossible, it can be interpreted as a simplification of an equilibrium mechanism. The equilibrium constant K is the ratio of both forward and backward reaction. With regard to temperature behaviour this equilibrium constant K can therefore be treated as a normal rate constant. The activation energy, however, is the difference between the activation energies

of forward and backward reactions. This difference can surely be negative if, for example, the backward reaction exhibits a stronger temperature dependence than the forward reaction. This is what happens with low temperature denaturation.

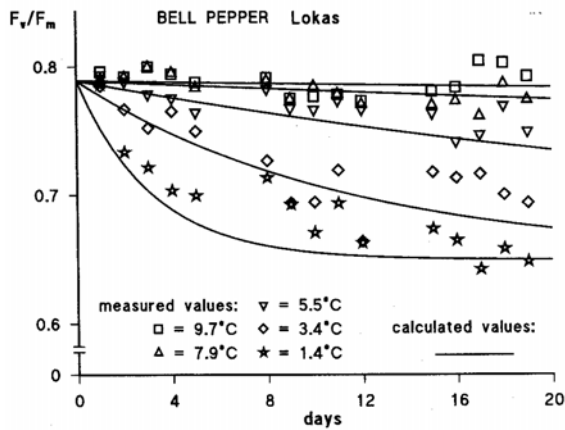


Figure 9-1 Quantum efficiency decay at different time - temperature combination for Bell pepper cv Lokas.

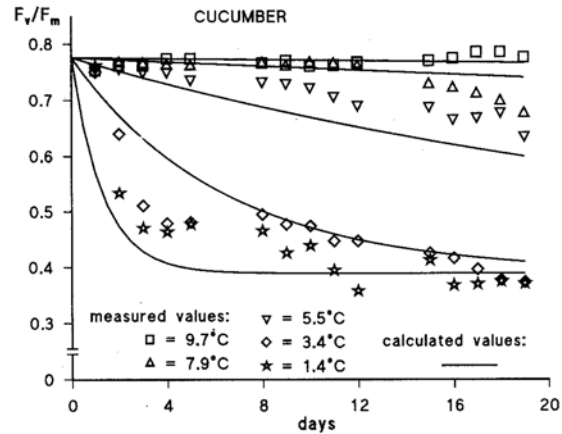


Figure 9-2 Quantum efficiency decay at different time - temperature combination for cucumbers.

## Results

### Time and temperature dependence of $F_v/F_m$ .

The measured quantum yield of PS II in both cucumbers and bell peppers shows a multiple exponential decay in time at different temperatures (Figure 9-1 to Figure 9-4). At 10 and 12 °C no chilling injury was detected during the experiment. The reference temperature ( $T_{ref}$ ) was therefore fixed at 10 °C or 283 K. At 6 and 8 °C the decrease in quantum yield corresponds to the development of visual chilling injury symptoms after the subsequent storage at 20 °C (compare Figure 9-1 to Figure 9-4). At 2 and 4 °C the decrease in quantum yield starts within the first 48 hours of cold storage though this does not result in any detectable chilling injury immediately after storage and with only slightly injury developing after 7 days at 20 °C. This is in accord with the results of Hariyadi and Parkin (1991) who found a significant reduction in reduced glutathione and  $\alpha$ -tocopherol after 2 days of storage at 4 °C (their first measuring point in time), while the detectable chilling damage, determined

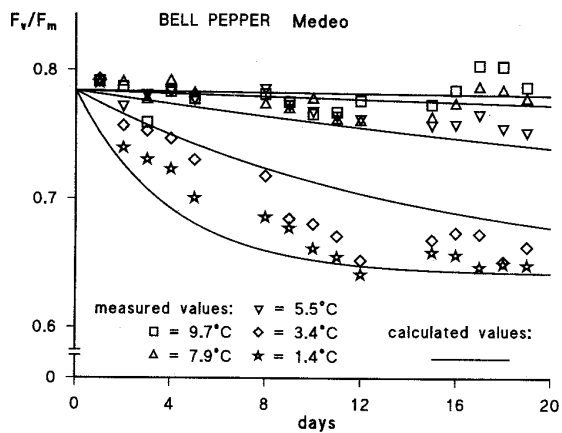


Figure 9-3 Quantum efficiency decay at different time - temperature combination for Bell pepper cv Medeo.

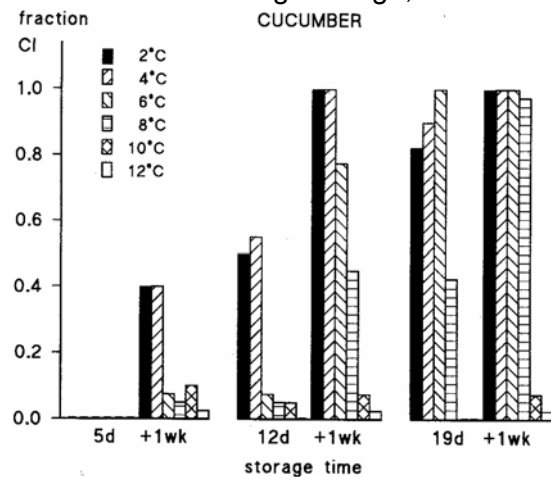


Figure 9-4 Fraction of fruits showing chilling injury at different time - temperature combination in pre and post storage for cucumbers.

as membrane leakage or ethane evolution, occurred after 5 to 7 days storage at 4 °C. Similarly, experiments performed on leaves of chilling sensitive cucumbers stored at 4.5 °C revealed 75% inhibition of O<sub>2</sub> evolution within 2 days of storage (Saczynska 1993). Our data of the visually assessed injury are in accord with these data (Figure 9-4). The behaviour of  $F_v/F_m$  is consistent with the described scavenger deactivation (Eq. 10) in combination with Arrhenius' law (Eq. 9). It is therefore proposed that the quantum yield of PS II, as measured with chlorophyll fluorescence, expresses a process which parallels the inhibition of the free radical scavenging system in the chloroplasts. Gounaris et al. (1992) have reported that the destruction of PS II in darkness is triggered by the amount of free radicals. It is possible that quantum yield values can be utilized as a measure for the number of active scavengers in chilling stressed systems. The  $F_v/F_m$  values however do not decay to zero. So, Eq. 10 has to be slightly modified to accommodate for this behaviour. The remaining minimal level of  $F_v/F_m$  is different for cucumbers and bell peppers, see  $Eff_{min}$  in Table 9-1.

$$Eff = Eff_{min} + (Eff_{max} - Eff_{min}) \cdot e^{-k_d \cdot t} \quad \text{Eq. 14}$$

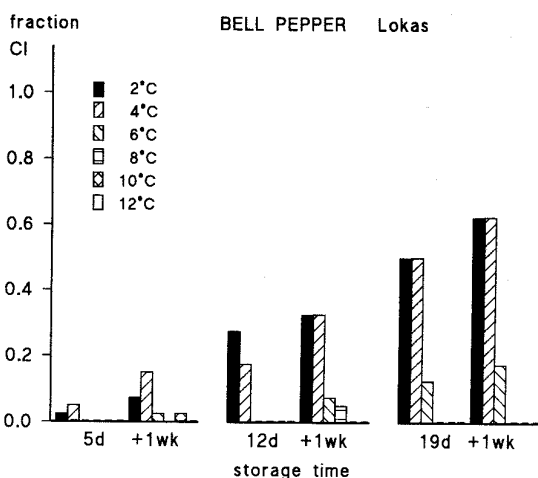


Figure 9-5 Fraction of fruits showing chilling injury at different time - temperature combination in pre and post storage for Bell pepper cv Lokas.

For the statistical analysis the average value for the efficiency values of 10 fruits was calculated for each experiment. The data sets of all efficiency values at each temperature-time combination, were combined giving a total of 360 data points.

This grand set was analyzed as a whole using nonlinear regression, based on Eq. 9 and Eq. 14 as a special case of Eq. 10.

The estimation results are shown in Table 9-1 In Table 9-2 the measured and calculated data are shown. Whether or not the activity of the radical scavengers is to be represented by the total  $F_v/F_m$  range, or only the variable  $F_v/F_m$  range cannot be concluded from this model and data set as  $k_r$  and  $Z$  appear as a product in each equation and cannot be separated.

### **Time temperature dependence of chilling injury.**

Eq. 12 describes the amount of radicals present in the system immediately after the cold storage. To describe the effect of temperature and time not only during cold storage but also during the subsequent post storage conditions, this equation has to be adapted slightly. By using the same equation again on the post storage condition (index w) with cold storage values (index c) as initial estimates, and assuming no further scavenger decay will develop at

Table 9-1 Chlorophyll fluorescence scavenging activity

	Cucumber Jessica	Bell Pepper Lokas Medeo	
		estimates	
$Eff_{max}$	0.77598	0.78918	0.7841
$Eff_{min}$	0.38886	0.64934	0.64112
$k_{dref}$	0.001058	0.001533	0.00111
$E_d/R$	-59098	-47881	-48828
$R^2_{adj}$	88.5	82.8	84.5
$N_{obs}$	360	360	359
	standard error of estimate		
$Eff_{max}$	0.00438	0.00178	0.00156
$Eff_{min}$	0.0077	0.00453	0.00495
$k_{dref}$	0.000234	0.000382	0.000279
$E_d/R$	2664	2841	2831
	standard error of estimate in %		
$Eff_{max}$	0.56	0.23	0.2
$Eff_{min}$	1.98	0.7	0.77
$k_{dref}$	22.12	24.92	25.14
$E_d/R$	4.51	5.93	5.8



the then higher temperatures, a final equation can be derived describing the formation or elimination of free radicals under cold and subsequent warm storage conditions:

$$\frac{R}{R_0} = e^{\left( k_{s\_c} \cdot S \cdot t_c + k_{r\_c} \cdot Z_0 \cdot \left( \frac{e^{-k_{d\_c} \cdot t_c} - 1}{k_{d\_c}} \right) \right)} \cdot e^{\left( \left( k_{s\_w} - S \cdot k_{r\_w} \cdot Z_0 \cdot e^{-k_{d\_c} \cdot t_c} \right) \cdot t_w \right)} \quad \text{Eq. 15}$$

The values for the remaining scavenging activities ( $Z_0 \cdot e^{-k_{d\_c} \cdot t_c}$ ) are predicted values based on the efficiency values previously estimated (see Table 9-1).

The combined data from each of the experimental combinations of time and temperature were analyzed with nonlinear regression based on Eq. 13 and Eq. 15. In the regression analysis the duration and temperature of storage under both cold and warm storage conditions, as well as the measured efficiency predictions, are used as independent variables. The fraction of fruits showing chilling injury is used as dependent variable. As it is virtually impossible to measure the amount of free radicals, let alone to determine their generation or scavenging, the amount of radicals is estimated as an intermediate, as required by Eq. 13.

The estimates of the parameters are shown in Table 9-2. In Figure 9-7 chilling injury is shown as a fraction versus estimated relative amount of radicals. The  $R^2_{adj}$  or percentage variance accounted for is 98% for cucumbers and 86% for bell peppers.

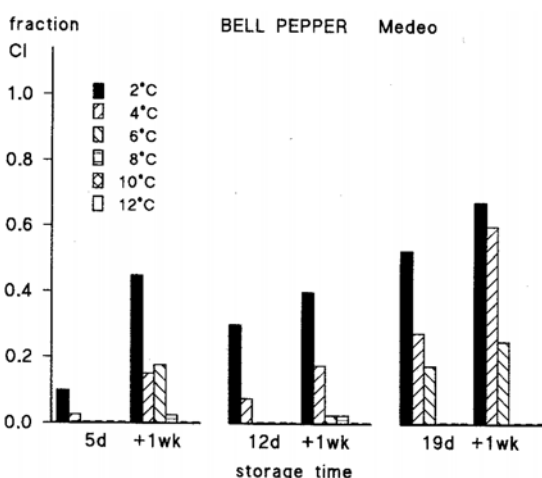


Figure 9-6 Fraction of fruits showing chilling injury at different time - temperature combination in pre and post storage for Bell pepper cv Medeo.

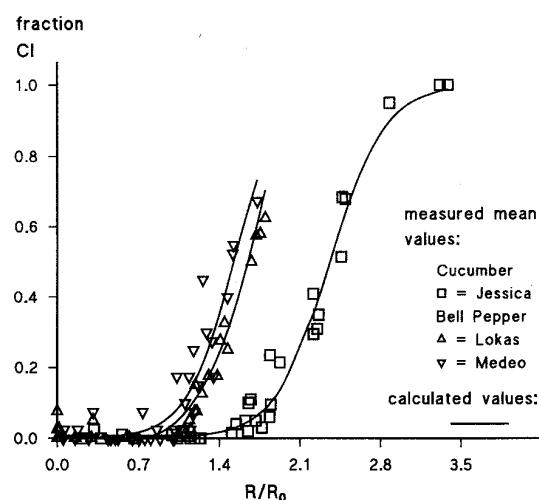


Figure 9-7 Fraction of fruits showing chilling injury versus estimated relative amount of free radicals (all data combined).

## Discussion

### Statistical analysis.

The interpretation and analysis of the efficiency data is relatively straightforward (see Table 9-1). A first order decay or denaturation of enzymes and scavengers increases with decreasing temperature (Eq. 9 and Eq. 10), showing a negative activation energy  $E_d$ . As already explained, this may imply an equilibrium reaction between an active and an inactive state of the enzyme. This equilibrium could explain the observed recovery of the efficiency after the cold storage period. This recovery is not included in the model nor the analysis. The calculated  $R^2_{adj}$  of about 85% for 360 measurements, indicates a rather good fit of the model presented to the data. The estimated standard error of estimates (standard error of estimate in Table 9-1) are also well within an acceptable range of 1 to 5%. The least reliable estimation seems to be the reference denaturation rate  $k_{dref}$ , indicating measuring errors to

be the main source of the remaining variance and not modelling errors. It also indicates that efficiency measurements can be used as an alternative for the direct assay of the amount of scavengers present.

The analysis of the measured data concerning the development of chilling injury is hard to interpret, as it is essential to include the mutual interference between time and temperature in pre- and subsequent postconditioning. In Table 9-2 the estimated parameters are shown for the mean value of chilling injury ( $N_{\text{obs}}=36$ ) as well as for the separate data ( $N_{\text{obs}}=144$ ), assuming the scavenging activity must be described by the dimensionless range  $((\text{Eff} - \text{Eff}_{\text{min}})/(\text{Eff}_{\text{max}} - \text{Eff}_{\text{min}}))$  and not by the actual range of Eff (see Eq. 14). Based on the preliminary results of statistical analysis, the parameter  $C_{\text{Cl}}$  is assumed to have a constant value of 10. For the estimates based on mean data, the percentage variance accounted for is remarkably high. Even for the separate data analysis, it is still very acceptable, showing more deviation in the bell peppers data, the product less sensitive to chilling injury. The estimates, as well as the deviation between them, for the different analyses and cultivars, and the standard error of estimates, indicate the radical decay reaction (Eq. 3) to be rather constant in temperature and over the cultivars:  $E_s$  seems to be zero while  $k_{\text{sref}}$  has roughly the same value for Cucumber and Bell pepper Medeo. Cultivar Lokas evidently displays a different behaviour. The estimate for  $k_{\text{Cl}}$  is, notwithstanding the assumed constant value for  $C_{\text{Cl}}$ , remarkably constant over the different products and cultivars.

Although a good statistical fit is by no means a validation of the proposed model, it certainly supports the veracity of the model. When estimating every parameter in the model, the results indicate an uncertainty in estimation: the standard errors of the estimates can not always be calculated. The reduction of the number of parameters to be estimated is one of the reasons for assuming  $C_{\text{Cl}}$  to be constant in the analysis. The uncertainty is interpreted by the statistical package as insufficient data to estimate each temperature-time relationship. The scavenging reaction in particular is suspect: the estimate of  $E_r$  is so high that no scavenging can take place at low temperatures. As there is only one high post storage temperature, the insufficiency of data for correct temperature dependence is explicable: the statistical systems tries to estimate a temperature relation ( $E_r$ ) with only one effective temperature.

Table 9-2 Fraction chilling injury vs amount of radicals

	Mean datapoints			Datapoint separate		
	Cucumber Jessica	Bell Pepper Lokas	Medeo	Cucumber Jessica	Bell Pepper Lokas	Medeo
	estimates			estimates		
$E_s/R$	1389	688	-1054	-1769	-3082	-895
$k_{\text{sref}}$	0.04838	0.03083	0.0213	0.05286	0.0209	0.02088
$k_{\text{rref}}$	0.04149	0.855	0.091	0.05057	0.594	0.0797
$E_r/R$	46706	73005	26735	36920	65956	20407
$k_{\text{Cl}}$	-4.236	-4.778	-4.916	-4.731	-4.798	-4.99
$R^2_{\text{adj}}$	97.9	96.4	85.3	88.5	70.9	62.5
$N_{\text{obs}}$	36	36	35	144	144	144
	standard error of estimate			standard error of estimate		
$E_s/R$	210	1292	1105	375	2256	1003
$k_{\text{sref}}$	0.00403	0.00402	0.00327	0.00649	0.00521	0.00295
$k_{\text{rref}}$	0.00551	0.165	0.0454	0.0074	0.283	0.0314
$E_r/R$	6311	1531	9831	6564	4675	7254
$k_{\text{Cl}}$	0.307	0.161	0.271	0.395	0.26	0.251
	standard error of estimate in %			standard error of estimate in %		
$E_s/R$	15.12	187.79	104.84	21.2	73.2	112.07
$k_{\text{sref}}$	8.33	13.04	15.35	12.28	24.93	14.13
$k_{\text{rref}}$	13.28	19.3	49.89	14.63	47.64	39.4
$E_r/R$	13.51	2.1	36.77	17.78	7.09	35.55
$k_{\text{Cl}}$	7.25	3.37	5.51	8.35	5.42	5.03

The activation energy for the radical reaction  $E_s$  is almost zero. This implies an almost temperature independent reaction rate, which is not unusual for radical reactions. Apparently, the model fits better to averaged data than for individual data. This implies a relationship with batches of product, rather than individual fruits. This is not at all surprising, as the conversion from intensity CI to fraction CI implies a population approach.

### ***Interpretation of the parameters and dynamic behaviour.***

Photosystem 2 quantum yield is assumed to reflect the activity of radical scavengers. This is based on comparison of the behaviour of  $F_v/F_m$  values with the behaviour of radical scavenging concentrations determined by Hariyadi and Parkin (1991). It is also to be expected that PS II quantum yield relates inversely to the initial concentrations of radicals as it has been found to be extremely susceptible to damage caused by active oxygen species (Gounaris and Selkirk 1992). The magnitude of the activation energy ( $E_d$ ) directly reflects the product susceptibility to chilling injury, including the relative range in temperature between start and completion of chilling injury. The magnitude of the reaction rate determines the temperature at which chilling injury will start to occur (Table 9-1).

The scavenging reaction determines, among others, the length of time a product can endure a certain temperature, provided enough scavengers remain from the deactivation system.

Regarding Eq. 15, the successive exponential terms can be interpreted roughly as follows: (i) The first term describes the actual development of chilling injury. (ii) The second term describes the cumulative history of potential chilling injury. (iii) The third term describes the future development of chilling injury. (iv) The last term describes the actual scavenging in the system.

Whether or not chilling injury will occur depends on the relative value of each of the terms. If the total amount of actual radical scavenging is larger than the total amount of radicals formed, only a limited amount chilling injury will develop. Otherwise chilling injury will develop quite explosively. The threshold value is defined by putting  $dR/dt$  equal zero in Eq. 7. This results in the following expression:

$$R_{eq} = \frac{k_i}{k_r \cdot Z - k_s \cdot S} \quad \text{Eq. 16}$$

Without an influx of radicals, generated by other processes than described by the model and neglected in the static model, the time-temperature combination for the threshold value, accumulated in  $Z$ , can not be estimated.

The dynamics of the model are quite useful in and capable of calculating the effects of varying temperatures during storage. Cold storage after several hours of prestorage at higher temperatures has been found to result in less severe chilling injury effects than cold storage directly after harvest (Abe 1990; Saltveit et al. 1987, Biesheuvel et al. 1992). Due to the large value of the activation energy of the radical scavenging reaction ( $E_r$  in Table 9-2), and a subsequent high scavenging activity, a prestorage period at 20 °C after harvest at low temperatures allows for a substantial reduction in radical concentration in the tissue at the start of the cold storage period, delaying the onset of significant chilling injury.

Although the number of different products researched is too small as to divide the parameters into product and process oriented groups, it is felt that this should be possible. Current results strongly suggest that the constants of the radical reaction ( $E_s$ ,  $k_s$ ,  $C_{Cl}$  and  $k_{Cl}$ ) are more linked with the chilling process itself, while the constants  $E_r$ ,  $k_r$ ,  $E_d$ ,  $k_d$  and  $Eff_{min}$  are more product oriented.

## **Acknowledgement**

This study was conducted in the framework of a research program on fruits and vegetables, partly financed by the Dutch Commodity Board for Vegetables and Fruits.

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Table 9-3 Legends and abbreviations to the equations.

Variable	Meaning
Cl	intensity of chilling injury
E	activation energy
Eff	efficiency
k	reaction rate constant
R	amount of free radicals
S	amount of substrate for chilling injury (double bonds in fatty acid chains)
t	time
T	temperature
Z	amount of radical scavenging enzyme
index	
abs	absolute (temperature)
act	actual value
c	cold or pre storage
Cl	chilling injury
d	denaturation
i	any index
max	maximum value
min	minimum value
na	not active
r	radical scavenging
ref	reference (temperature)
s	substrate
w	warm or post storage
0	initial amount



10

**Modelling the firmness of Elstar apples**

**during storage and transport**

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Lecture held at the International Symposium on *Effect of Preharvest and Postharvest Factors on Storage of Fruits and Vegetables*, August 3-7, 1997, Warsaw, Poland. *Acta Horticulturae* **485**, 363-372

## Abstract

A dynamic model has been developed that describes the decrease in firmness of Elstar apples during different types of conditions, based on the general knowledge how firmness is affected by chemical and biochemical reactions, and on the strict and consistent application of fundamental kinetics.

The variable part of the firmness of apples is deduced to depend mainly on the pectic compounds in the middle lamellae. These pectic compounds are supposed to decay during storage in two distinct routes, one consuming / needing oxygen and one occurring with or without oxygen.

Depending on the gas conditions (oxygen and carbon dioxide) the respiration rate of apples changes. This respiration affects the rate of the oxygen consuming pectin degradation. The moment of harvest defines among others, the initial climacteric stage of the apples. Depending on the relative respiration rate, the rate of development of the climacteric stage changes. The climacteric stage in itself affects the rate of pectin decay: preclimacteric apples can be kept longer than climacteric ones.

During CA or MA storage, an enzyme, probably polygalacturonase, accumulates depending on the length of the CA/MA period: the longer kept at CA/MA conditions, the faster firmness decays at the end of the storage period.

On top of all those interactions, a temperature dependence is applied on all individual reaction rates. This temperature dependence is described by the well know Arrhenius law.

The model can be applied to predict the firmness of Elstar apples during all kinds of scenarios encountered in practice, to get an impression of the expected final firmness when reaching the consumer.

## Introduction

The behaviour of firmness of apples is and has been subject of study for already a long time. Knowledge about this behaviour has been emerging from these studies almost as long. Compilations of this kind of information invariably result in very informative and long monographs (e.g. Thompson 1996). This knowledge has, however, never been interpreted using one consistent and multidisciplinary philosophy. In this chapter a model is described, attempting to combine the knowledge available into a mathematical and dynamic formulation. The effects covered by the model include time of storage, temperature and ethylene during storage, as well as the effect of harvest maturity. The model is not validated in the classical sense of the meaning, but checked against the expert opinion of long time apple storage and quality researchers.

## How is firmness built up?

The quality attribute firmness of agricultural products can be regarded as the way human beings can detect the physico-mechanical properties of these products. The texture of agricultural products is built up by a combination of the physical forces originating from the following processes or properties that generate strength upon compression or chewing:

- turgor pressure inside intact living cells, creating a tissue tension
- special compounds / granules inside the cell possibly generating strength (e.g. starch)
- cohesive forces within a cell, generated by chemical composition and physical properties of the cell wall
- adhesive forces between cells, generated by chemical composition and physical properties of the middle lamellae and the pectin chains
- overall structure and shape of separate cells (cell dimension and contact area)
- overall structure and shape of tissue, like strength and distribution of vascular tissue

In this overview, the first four items represent the chemical and physical based forces, the last two items represent the histological and morphological ones (archestructure). Within the textural behaviour of a particular fruit, all these aspects are more or less present and heavily interacting. Depending on relative occurrence and importance of the mentioned items, a very



diverse range of observed behaviour of texture and firmness can be depicted, e.g.:

- with only turgor or tissue tension as the major item, products are soft and juicy, losing texture upon processing like strawberries
- with pectin forces overruling, products are essential crispy and juicy (rupture through cells with release of juice from the disrupted cells) like fresh apples
- with cell wall forces overruling, products are essential mealy and dry (rupture along cells without release of juice from the intact cells) like sometimes senescent apples
- with vascular tissue important, products are essential tough and fibrous like sometimes in asparagus

## How does firmness change?

Each process and each property mentioned in previous paragraph can and will have its effect on the observed firmness of any product, including apples.

**Structural aspects** and special compounds inside the cell are predominant in determining the type and the level of firmness in any fruit, but will most probably not (much) change during storage (e.g. cell shape and elasticity of the primary wall) as can be taken from the same firmness remaining after storage at different temperatures (see Figure 10-1 Thompson 1996). These structural aspects exhibit a cultivar specific but standard developmental pattern during growth (Fry 1988).

**Pectin** may decay by enzymatic action of pectin methyl esterase (PE) and of polygalacturonase (PG) (Keijbets 1974, Voragen & Pilnik 1989). PE activity decreases the degree of methylation of the pectin chain (Rodis et al. 1997, Tijskens et al. 1997a, 1997b). PG decreases the length of the pectin chain (Tijskens et al. 1998). So, both enzymes primarily affect the middle lamellae and hence the strength of the cell-cell adhesion.

**Water loss** will affect the turgor and the tissue tension.

So, during storage, firmness will only change by the last two processes. The invariable properties are induced by cellulose composition of the cell wall, overall cell size, cell structure, vascular tissue and special compounds inside the cells. They most probably do not change much at normal storage conditions. They contribute, however, for the major part to the existence of the firmness remaining even after prolonged storage (Tijskens 1979).

A model describing firmness behaviour of apples has consequently to be founded in a vast simplification, with as little violation of the real world system as possible. It will not be as comprehensive on a detailed compositional basis as, e.g. Carpita & Gibeaut (1993). It has, however, to be much more comprehensive with regard to completeness of important processes involved in a multidisciplinary approach, combining physical, chemical and biochemical effects on the observed firmness. The rates of the 'selected' processes will depend on the actual conditions during ripening and storage with respect to temperature, relative humidity, gas conditions (O<sub>2</sub>, CO<sub>2</sub>, ethylene) and the actual levels of several enzyme activities.

The effects of water loss on the firmness of apples will not be further discussed in this paper.

## Model formulation

The basic processes occurring during growth (Sfakiotakis and Dilley 1973), included in the mathematical model, are:

- ripening at the tree including the on tree ripening inhibition process.
- development of climacteric stage

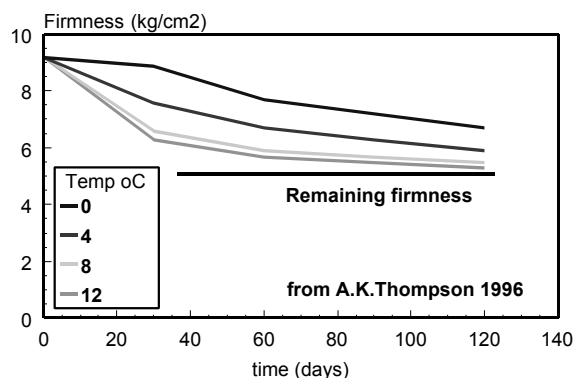


Figure 10-1 Pattern of behaviour of apple firmness during storage at different temperatures.

As there is not much information on the exact nature and mechanisms for these processes, which are actually the same, they are described by an empirical logistic (sigmoidal) curve. This reflects roughly the general experience with respect to ethylene dose-responses and on-tree ripening (Lau et al. 1986, De Pooter and Schamp 1989).

The basic processes included in the mathematical model occurring during storage are:

- pectine decay affected by O<sub>2</sub>
- pectine decay without effect of O<sub>2</sub>
- moisture loss

The two types of pectin decay are generally not recognised as such in literature. Tijssens indicated already in 1979, that the general pattern of firmness decrease could be regarded as the result of two pectin decay processes (eq. 1).



Formulation of the mechanism as shown, does not imply that this is the exact nature of the processes going on in the fruit, which is probably a system of enzymatic reactions involving PE and PG, but merely that the phenomena observed can be explained, by simplifying the actual processes into this mechanism. The first one always occurs, regardless of the gas conditions applied, the second process needs oxygen to proceed, and will consequently be slowed down by CA conditions (see the paragraph: Effects of CA conditions).

## Effects of temperature

All rates of chemical and biochemical reactions depend on temperature. In the model formulations, the relation given by Arrhenius' equation is used throughout for each reaction rate constant:

$$k_i = k_{i,\text{ref}} \cdot e^{\frac{E_a}{R} \left( \frac{1}{T_{\text{ref}}} - \frac{1}{T} \right)} \quad \text{eq 2}$$

This not only includes the pectin degrading reactions, but also the effects of ethylene, respiration, ripening (climacteric stage) and senescence. The energy of activation (E<sub>a</sub>) is a measure for the susceptibility of that reaction for temperature. Each reaction rate constant has its own specific dependence on temperature, expressed in the energy of activation. This explains the sometimes observed difference in relative importance of different reactions / processes at different temperatures.

## Effects of CA and MA conditions

Applying low oxygen and high carbon dioxide levels in the storage rooms is already long time recognised as a valuable tool in increasing the storage potential or keeping quality of apples. It is generally assumed that it acts by slowing down (almost) all chemical and biochemical reactions occurring in the product. According to Solomos and Kanelis (1989) and Kanellis et al. (1993) the effect of oxygen level or respiration intensity is different for different reactions. There is, however, not much numerical information on this subject. Recently, some models came available (Peppelenbos et al. 1993, 1996, Hertog et al. 1997) that describe the intensity of the respiratory processes as a function of O<sub>2</sub>, CO<sub>2</sub> and temperature. The general

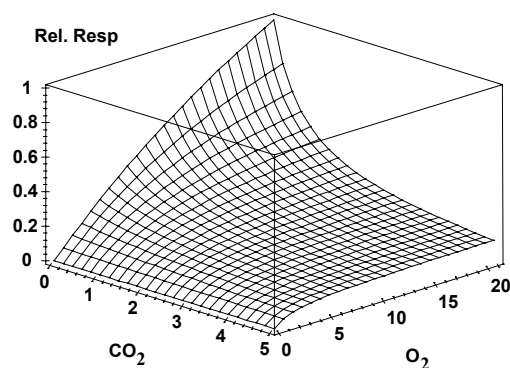


Figure 10-2 Relative respiration in Elstar apples as simulated by the model

formulation was used to develop, for aerobic situations, a relative respiration (Tijskens 1995). Relative respiration is the ratio of the respiration intensity at any temperature and gas condition, to the respiration intensity at the same temperature in air.

$$\text{RelRe sp} = \frac{\text{O}_2}{21} \cdot \frac{1 + K_{\text{mo}} \cdot 21}{1 + K_{\text{mo}} \cdot \text{O}_2 + K_{\text{moc}} \cdot \text{O}_2 \cdot \text{CO}_2} \quad \text{eq. 3}$$

This relative respiration proved to be more or less independent of temperature, and can consequently be used to separate the effects of gas condition from the effects of temperature. In Figure 10-2 an example is shown for the behaviour of relative respiration as a function of the applied O<sub>2</sub> and CO<sub>2</sub> levels in Elstar apples. The relative respiration is used in the model to modify the rates of some of the processes like that type of pectine decay that needs oxygen, and the development of the climacteric stage.

During CA and/or MA storage a second process is occurring. The longer CA storage takes places, and the more intensive (the lower the relative respiration) the faster firmness decay will be when putting the product again at normal conditions (air and room temperature). This is modelled as the accumulation of an enzyme (maybe PG, more probably related to ACC production and accumulation) that enhances pectin decay in the ex-store life.

$$\frac{\partial \text{PG}}{\partial t} = k_{\text{PG}} \cdot \text{PG} \quad \text{eq. 4}$$

The oxygen induced decay of apple pectin than becomes:

$$\frac{\partial \text{Pect}_{\text{pO}}}{\partial t} = -k_{\text{pO} \cdot \text{Pect}_{\text{pO}}} \cdot \text{PG} \cdot \text{RelRe sp} + k_{\text{pO}} \cdot \text{Pect}_{\text{pO}} \cdot (1 - \text{RelRe sp}) \quad \text{eq. 5}$$

This formulation, however, is too empirical and most probably an incorrect representation of the processes involved. Studies to correct this situation are being conducted.

## Effects of harvest maturity & ethylene

The effect of ethylene on pre- and postharvest behaviour of apples has been modelled with an empirical function. It is assumed that ethylene induces ripening at the tree and during storage pretty much in the same way, except for the on tree ethylene inhibition. Production of ethylene is modelled as the derivative of the well-known logistic curve:

$$\frac{\partial \text{Eth}}{\partial t} = k_{\text{Eth}} \cdot \text{Eth} \cdot \left(1 - \frac{\text{Eth}}{\text{Eth}_{\text{max}}}\right) \quad \text{eq. 6}$$

The action exerted by this amount of ethylene is proportional to the integral of this function, again taking the variable temperature during all kinds of actions into account. The time in this equation is somewhat ambiguous. In postharvest research and postharvest practical applications, we are used to start counting the time from the moment the food chain begins, which is usually the moment of harvest. To obtain an initial level of ethylene, necessary with this oversimplified logistic function, the moment of harvest has to be expressed relative to a fixed point in time, let's say the optimal harvest time.

The climacteric stage of the product, both preharvest and postharvest, depends on the history of ethylene production and action. On his turn, the climacteric stage, expressed as a fraction of the maximal climacteric stage, affects a number of processes like pectin decay.

## Chain simulation, a practical application

The model describes the behaviour of firmness based on the processes possibly occurring in any part of the lifespan of an apple, from growing over harvest to storage and transport. The model can now be applied to predict the firmness in any known or unknown scenario of temperature and applied gas conditions, from any starting point with regard to maturity at harvest or growing condition.

Optimisation of complete chains of product handling with respect to the expected firmness becomes feasible. The optimisation itself has, for the time being, to be conducted by hand. Compiling this model into a computer initiated optimisation requires a more validated model to permit such an effort.

In the next figures some examples are given for the prediction of firmness of Elstar apples during various scenarios and harvest maturities.

### **Example 1: variable temperatures**

In Figure 10-3 to Figure 10-6 a scenario was applied to apples with a harvest maturity of 18 days before climacterium, with in total 1.5 days at 20 °C in air to simulate harvest, transport and auction before commercial storage, followed by a commercial storage at 1 to 6 °C in an atmosphere of 1% O<sub>2</sub> and 3% CO<sub>2</sub> at a relative humidity of 90%.

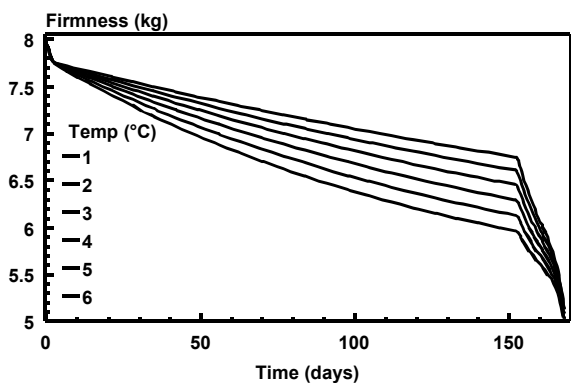


Figure 10-3 Firmness during storage and ex-store life at different storage temperatures

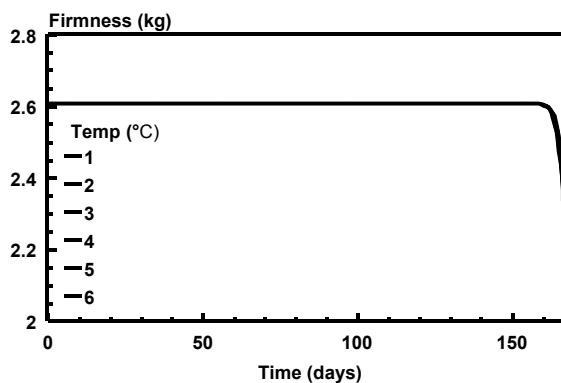


Figure 10-4 Firmness generated by oxygen sensitive pectin decay

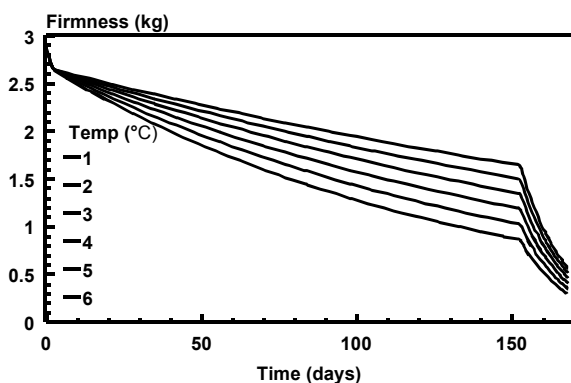


Figure 10-5 Firmness as generated by oxygen insensitive pectin decay

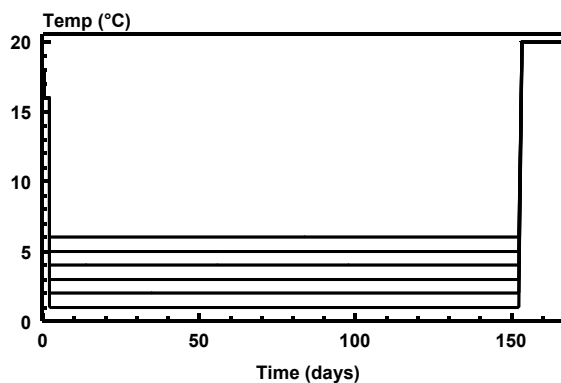


Figure 10-6 Temperature scenario during storage and ex-store life

The effect on firmness decay by different storage conditions can clearly be seen. Also it should be noted that the firmness only decreases by action of the pectin decay that does not need oxygen (Figure 10-4 and Figure 10-5). Also can be noted the (small) difference in rate of firmness decrease during the ex-store period of 15 days at 20 °C, which is a simulation of the shelf-life at the consumers.

### **Example 2: Variable harvest maturity**

In the same chain setup as in example one, apples with a harvest maturity from 25 to 0 days before reaching the climacteric stages, were stored at 1°C in 1% O<sub>2</sub> and 3% CO<sub>2</sub>. In the next figures, the simulated behaviour is shown.

The effects of harvest maturity are dramatically visible in Figure 10-9 showing the

development of the climacteric stage. The riper the apples are at harvest, the sooner climacterium is reached, even in CA conditions. The effects on firmness during storage are not that dramatic, but the firmness decreases considerably faster during the shelf-life of the product the riper the apples were harvested.

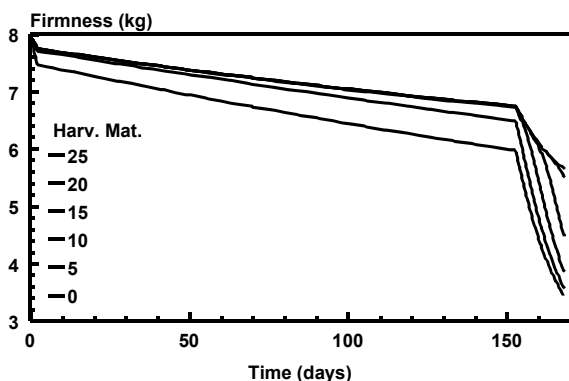


Figure 10-7 Firmness as affected by maturity at harvest

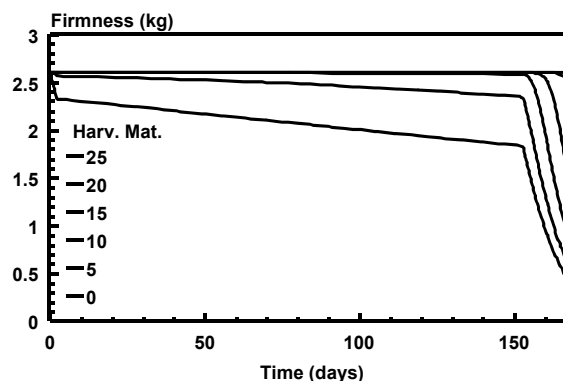


Figure 10-8 Firmness generated by oxygen sensitive pectin decay

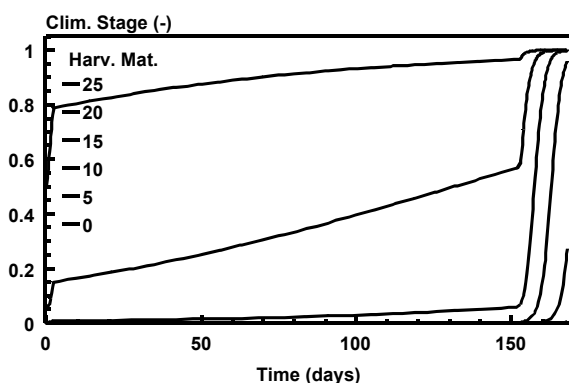


Figure 10-9 Development of climacteric stage for different maturities at harvest

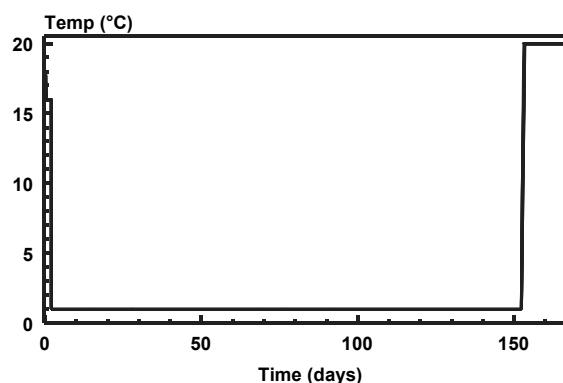


Figure 10-10 Temperature scenario for apples with different maturities at harvest

## Conclusions

Predictions can be made by mathematical simulation of the behaviour of firmness of Elstar apples during all kinds of storage and transport scenarios. The model structure is more based on the processes involved than on the observed phenomena. The modular structure of the model provides information about the effects of the conditions applied in the chain on the individual constituting parts of firmness.

It should be noted that using modelling, the exact prediction of firmness itself is very difficult. The major advantage of simulation research is to be found in indicating trends in product behaviour upon a change in scenario applied.

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## **Part 4**

# **PRODUCT BEHAVIOUR IN PROCESSING**

## Introduction

The purpose of processing of fruits and vegetables is primarily targeted at increasing the economic lifespan of these perishable products. Since the major cause of product deterioration in the fresh, unprocessed state is either physiological or bacterial in nature, processing acts primarily on inactivation of enzymes to block physiological changes and the partial (pasteurisation) or complete (sterilisation) removal of bacterial individuals to block bacterial deterioration. The price that has to be paid for applying these high intensity processes is a simultaneous decrease of various quality attributes and hence overall quality. The type of attributes for processed products does in fact differ not too much from the ones described for fresh produce (see introduction Part 3).

The classical techniques are mainly based on heat processing. At the temperatures usually applied massive quality losses are induced. The main principle upon which the high temperature processing is based, is the fact that the temperature sensitivity (actually the activation energy) is much higher for bacterial killing, than for enzyme denaturation than for quality deterioration. Since a twelve fold decimal reduction of bacterial spores is the processing target prescribed by legislation (the so-called  $F_0$ ), more quality is retained during high temperature processing, but also somewhat more enzymes may remain active. In Fig Part 4-1, a simulated example is shown for the % quality retained of the variable part of

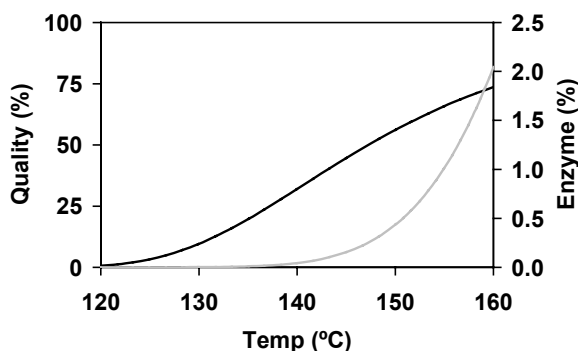


Fig Part 4-1 Example of remaining quality (black line) and enzyme activity (grey line) in classical heat processing as a function of processing temperature.

quality, at processing to an  $F_0$  value of 1 at increasing processing temperatures. The percentage remaining active enzyme is also indicated. The main problem with applying excessive high temperatures, is the increasing difficulty in controlling the temperature in each and every location in the product and processing chamber, coupled of course with the increasing difficulty of controlling time (at 145 °C the total processing time is about 20 s) and of cooling down the product rapidly enough to prevent over-processing.

Modern techniques for food processing apply technologies that rely less on temperature but more on other physical processes for inactivation of enzymes and killing of bacterial spores. One of the more promising techniques is High Pressure Processing. Besides an effect of temperature, also an effect of applied high pressure on the sensitivity of all kind of reactions is utilised. Most probably those reactions are favoured by the decrease in the specific volume of the total amount of reactants. Apparently bacteria and enzymes are again more sensitive than ordinary chemical reactions. The complexity of modelling this kind of processes rapidly increases to a level one can hardly understand what exactly is going on. But still the principle of problem decomposition into the constituting processes, and application of fundamental rules apply even in these complex interactions (Hendrickx 1998, Indrawati 1999).

Another promising technique is the cold plasma inactivation of bacteria and spores. This technique rather targets at removing bacterial life forms without altering too much the product enzymes included (Bogaerts et al. 2002).

With respect to quality deterioration during processing, all techniques involve denaturation of enzymes, combined with the exerted action of these enzymes during processing. Since the killing of bacterial cells and spores during processing is rather straightforward to model (first order mechanism), main emphasis will be put on the enzyme inactivation.

The essential difference with processes going on at conditions usually encountered during growth, distribution and storage, is the range of temperatures applied. When modelling the observed phenomena during different temperature ranges, one has to consider which processes become important at these higher temperatures that were unimportant at lower



temperatures, by the mere effect of the Arrhenius relationship. Also one has to consider which processes are no longer of any importance to consider, by the mere fact that the process has become so quick that all conversions have already taken place, before even measurements can be conducted. But even these considerations do not change the fact that all processes, whether or not important for a particular application, occur with the same mechanism and rate constant.

In this part, two examples will be worked out for the effect of the exerted action of PE on textural properties during blanching and subsequent cooking in carrots (chapter 11), and on physical process in the changes in colour of green beans as a consequence of blanching procedures (chapter 12).

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**11**

**Mathematical modelling of enzymatic reactions as related to the texture of fruits and vegetables after storage and mild preheat treatments.**

**BRAM: Blanching Response Amplification Model**

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Published as Chapter 8 In S.M. Alzamora, S.M. Tapia, A. López-Malo (eds.). *Minimal processed fruits and vegetables*, (2000) Aspen Publishers Inc. USA. p. 127-152.

## Introduction

The invention of the sterilisation process by Nicolas Appert almost two centuries ago, was a major break through in food processing. This process offered mankind the possibility to extend the keeping quality of perishable agricultural products during substantially longer periods than was ever optional for the raw materials as such.

In time it was however realised, that this process strongly affects quality attributes of the product like texture, colour and taste. Furthermore, this process negatively affects (hidden) product properties like for example the vitamin C content.

During the last three decades prolonging keeping quality has turned from art into science. The underlying reasons for this transition from art to science are related to:

- an improved basic understanding about plant physiology, plant genetics and (some of) their relations
- raw materials with improved properties: these properties are related to quality attributes like texture, taste, flavour and colour
- improved logistics and management of the raw material in a chain
- process technological developments, ranging from the improvement of existing technologies to the industrial implementation of not yet applied technologies.

In conjunction to these scientific and technological developments the perception of product quality acquired by the consumer is semi-continuously influenced by the mutually dependent and interacting parameters "product, consumer, market" (Sloof & Tijsskens, 1995).

At this very moment the consumer is very concerned about the fresh image of agricultural products, as related to a healthy image. In addition, the consumer is also spending less time to produce a meal. This implies an increase in both the "ease to use" of edible products and of convenience. As a consequence of these developments one can observe at one hand activities focused on the extension of the keeping quality of agricultural raw materials by processing these raw materials minimally. At the other hand, efforts are undertaken to reduce the impact of the sterilisation process on both product properties as well as quality attributes of the perishable products by adapting this process. In essence these two approaches are followed to deliver products to the market with an optimal "fitness for use" for the consumer (Kramer & Twigg, 1970; Steenkamp, 1989).

The contents of this chapter are focused on the effects of both temperature and time on the important quality attribute "texture" of fruits and vegetables. Information was gathered about specific enzymes, which are either known to, or assumed to affect texture, either during storage or heat treatments. A set of chemical reactions was defined based on this information, information from literature and generally accepted theories. In essence, these sets of chemical reactions are the core of the models. Using the well-known rules of chemical kinetics (Cabral *et al.* 1993; Segel, 1993; Whitaker, 1994), these models can further be developed into their analytical solutions. In other words underlying processes affecting texture were modelled rather than texture itself.

So far, two main items have been mentioned: texture and modelling. To obtain a better insight in the modelling approach chosen in relation to texture in first instance a verbal description of both texture and modelling will be given. Examples will be worked out and discussed making use of this "chemical kinetic" approach during either storage, or preheating treatments of fruits and vegetables.

## Texture and texture generating forces; a verbal model.

### *Global origin of texture*

Considering the origin of texture three levels of abstraction can be discerned: the molecular level, the cellular level and the organ level.

At the molecular level, the key determinants of texture are the chemical nature of the plant cell wall and the interactions between the constituting biopolymers. During the development and senescence of plant organs, modification of cell wall polymers results in a change in the contribution of textural perceived properties. Compare for example the perceived texture

between an immature, a mature and an overmature apple. The majority of these changes in the raw material have been attributed to cell wall metabolism, either during ripening (Martin-Cabrejas *et al.*, 1994; Luri *et al.*, 1994; Seymour *et al.*, 1990) or storage (Harker & Hallett 1992). Such changes can lead to a deterioration in texture and palatability of plant organs as a result of over-softening or toughening. Even in the best studied example of a pectin degrading enzyme, polygalacturonase, no firm conclusions have been reached on its role in the softening of fruit during ripening (Smith, 1988). It is, however, generally accepted that these cell wall modifying enzymes contribute to the texture of the final product, whether this product has either been stored, or given a heat treatment. However, little information is available on their effect on the texture of either the storage, or the heating process and on the rate and the contribution of the chemical and biochemical alterations taking place in the cell wall.

At the cellular level, the key determinant of texture is the tissue architecture, which comprises cell wall thickness, cell size, cell shape, cell adhesion, and tissue organisation. For example, when cells become larger the surface to volume ratio decreases. Assuming that the surface relates to the cell wall, with its major contribution to texture, it can easily be anticipated that this increase in cell volume will have an effect on the perceived texture. When on the other hand, the cell-cell adhesion decreases this will also have consequences for the perceived texture. By applying a rupture force on a tissue with low cell-cell adhesion forces (the cells are not strongly glued together) the tissue will break between the cells and the cell contents retain within the cell upon fracturing of the tissue. When the cell-cell adhesion forces are high (the cells are strongly glued together) the tissue will break through the cells and the cell contents are liberated. So, the cell-cell adhesion not only has consequences for the perceived texture, but also for the perceived taste and flavour. The main locus of the cell-cell adhesion is the middle lamellae region.

Textural properties are measured at the organ level, by rheological measurements and/or perceived by sensory analysis. Nevertheless, the way textural properties change ultimately depends on effects exerted at the molecular level, affecting tissue architecture at the cellular level and will finally have consequences at the organ level.

Little information is available on the effects of either storage or heating on the molecular level and how these effects at the molecular level are translated to the cellular and organ levels and finally the perceived texture. The relations between (bio)chemical composition, physical appearance and mechanical properties are largely unclear. It is obvious that the textural behaviour of a product, either during storage, or a heat treatment cannot be described exclusively based on information from only one of these levels

Going from a molecular level, through the cellular level to the organ level a hierarchical interaction pattern is described. In addition to this hierarchical interaction pattern it is obvious that the texture of agricultural products is caused by a combination of physical forces originating from the following processes or properties:

1. turgor pressure inside intact living cells and the associated tissue tension ,
2. special compounds inside cells possibly generating strength (e.g. starch),
3. cohesive forces within a cell: chemical properties of the cell wall,
4. adhesive forces between cells: chemical properties of the pectin,
5. overall structure and shape of separate cells,
6. overall structure and shape of tissue: strength and distribution of e.g. stronger shaped vascular tissue.

In this summation items 1-4 represent the chemical and physical based forces and items 5-6 represent the histological and morphological ones (architecture).

Depending on relative occurrence and relative importance of one of the mentioned items, a very diverse range of textural behaviour can be depicted, e.g.:

- with only tissue tension (turgor) as major item, products are soft and juicy, losing texture upon processing, like fresh strawberries
- with pectin forces overruling, products are essentially crispy and juicy (rupture through cells; juice with contents comes out of disrupted cells), like fresh apples

- with cell wall forces overruling, products are essentially mealy and dry (rupture along cells; juice with contents stays inside intact cells), like sometimes in overripe apples
- with vascular tissue important, products are essentially tough and fibrous, like sometimes in asparagus

### ***Cohesive forces within a cell and adhesive forces between cells***

In their schematic model representation of the primary plant cell wall Carpita and Gibeaut (Carpita & Gibeaut, 1993) the cellulose microfibrils are embedded in, or surrounded by hemicelluloses. This cellulose-hemicellulose network, chemically more or less inert, provides the basis of the plant cell wall. Interwoven with this network are the pectic polymers. Pectin is the description for a family of hetero-polysaccharides, rich in (methylated) D-galacturonic acid, including polysaccharide side chains (Fry, 1986). Pectins are block polymers (Jarvis, 1984) which contain both linear homogalacturonan blocks ('smooth regions') and branched blocks of rhamnogalacturonan ('hairy regions') with neutral side chains (De Vries *et al.*, 1982). The knitwear of these three distinct polysaccharides forms the basis of the cohesive forces within a cell. Furthermore, it is assumed that the cellulose-hemicellulose matrix forms the stretch-resistant, load-bearing part and that the pectin matrix forms the compression-resistant part of this network (Mutter, 1997). In addition to their contribution to the cohesive forces, pectins are thought to be mainly responsible for the adhesive, cementing forces between cells. This cementing function of pectins is assumed to be mainly performed in the middle lamellae region between adjacent cells. However, it is not yet clear whether this cementing function is caused by 'egg-box' structures, induced by calcium complexing of unsubstituted homogalacturonans (Grant *et al.* 1973), or by neutral side chains of the pectic polysaccharide entangled in and with the cellulose-hemicellulose region causing intercellular adhesion (Kikuchi *et al.*, 1996).

### ***Relation between chemical properties of (bio)polymers and physical forces.***

The stereo configuration of biopolymers (curly like starch or linear like cellulose) has a major impact on their physical behaviour (strength and elasticity). This stereo configuration however, depends on polymer type only. For the cellulose-hemicellulose network the stereo configuration is considered to be constant for agro-products either when put into storage, or during a heat treatment.

The rupture strength of linear polymers is generally accepted to be proportional (for the chemical aspect) with the degree of polymerisation. Based on the assumption that pectin and cellulose are long linear polymers, decay of their chain length explains the loss of rupture force with a first order reaction: for each cut in each chain the remaining length is half its original (Saedt *et al.* 1991).

For linear polymers with sidechains, like pectins, the situation is somewhat more complicated. Although it is not quite clear whether short sidechains increase the intrinsic rupture strength of the complete chain, the embedding factor (e.g. root system in soil) is of major importance. The effect of sidechains on texture will depend less on DP (Degree of Polymerisation) than on spatial distribution and slipping inhibition.

For real three dimensional polymers the situation is even more complicated: on top of the increase in embedding force (exponential towards limit), for each crosslink existing between the same two polymer chains, one cut is necessary for an effective chain shortening to occur. So, decay can already take place a long time before any signs of decay can be observed on a physical basis (rupture). On the other hand, one new crosslink between unlinked chains generates a huge increase in the degree of polymerisation and hence in rupture strength. The spatial structure and the distribution of sidechains and crosslinks will therefore define the type of behaviour: too much crosslinks on a small volume does not really increase strength but improves brittleness; too few crosslinks generate linear polymers with high elasticity and low strength. So, for non-linear polymers, the relative position of the crosslinks is of major importance.

### ***Enzymes and texture***

Like any part of living plants, cell walls are continuously prone to orchestrated, enzyme

catalysed alterations of their chemical composition during growth, maturation and senescence. For green beans, during these sequential stages of growth, maturation and senescence not only the chemical composition of the cell walls changes, but also the amount of enzymes capable to react with cell wall polymers (Stolle-Smits, 1998). Huge efforts have been made to study the role of pectins and pectolytic enzymes in the softening of fruit tissue during ripening (Atherton, 1986). With regard to these texture modifying enzymes, it has to be realised that the enzyme activity at a given time is not the most determining factor for texture behaviour. It is the total exerted action of the enzyme activity over a given period of time that determines the chemical changes that have taken place in the cell wall polysaccharides and the subsequent physical consequences.

## **Mathematical modelling of enzymes and texture**

### ***Models and model levels***

Models are the mathematical description of a part of a real world situation. Two approaches with regard to modelling can be distinguished leading to either empirical models or more fundamental models. Empirical models are based on the (statistical) analysis of a relation between input and output data, a dose-response relation. Examples are exponential or logistic functions describing the softening of fruit, or Near Infra Red calibration curves predicting either chemical or physical parameters, or perceived texture attributes of agro-products. More fundamental models are based on kinetic mechanisms and fundamental laws, e.g. Arrhenius. Of main importance for these types of models is the basic understanding of the processes underlying the observed phenomena one wants to model, rather than the phenomena themselves (Tijssens *et al.* 1997c).

With empirical models one tries to integrate domain knowledge of specific products with a measuring technique to generate statistical based prediction models. It has to be realised that the empirical models do not add to the understanding of product behaviour. These empirical models are, however, in general well suited for practical applications since they have most of the time a high predictive power. In general these empirical models are limited to the measuring situation.

The information provided within this chapter focuses on the more fundamental models. These models can be divided into three distinct levels of dynamism. In increasing hierarchical order one can distinguish:

The static model gives a state description at a certain constant time. The static model states in fact the situation of the product, together with the relational functions between state and observed properties. It relies primarily on data from chemical analysis.

The dynamic model describes the product behaviour with time, but with otherwise constant environment (external factors). The dynamic model describes state changes in time. State changes in time refer for example to changes taking place during a heat treatment, or to changes upon storage. This type of model can (possibly) be formulated with algebraic function (analytical solution). In most cases, however, differential equations are necessary. The model needs information, not only about chemical compounds (type and amount), the enzymes acting upon them, (type and amount), but also the generation and degeneration in time of the enzymes involved. This dynamic model relies on the static model extended with enzyme data.

The supra-dynamic model describes product behaviour with time, and with changing environment (external factors), in other words it describes changes in states during time under changing external factors. It is impossible to formulate this type of model with algebraic functions (analytical solution), and one has to make use of differential equations. It needs the same kind of information as the previous (dynamic) type of model. On top of this all, the influence of the changing external factors upon these activities has to be known (estimated). It relies on the previous type of models, augmented with data of processing and its influence e.g. with a temperature process. This implies that activation energies are required to be known based on the assumption of an Arrhenius type behaviour.

### **Model development and statistical analysis**

The models were developed using a system of problem decomposition (Sloof & Tijskens, 1995). This system is oriented towards modelling of the underlying processes that cause the observed phenomena rather than the modelling of the observed phenomena themselves. The models are based on kinetic mechanisms describing the particular process. The models were developed further by using the well-known rules of chemical kinetics. The mathematical development and statistical analysis were carried out according to (Tijskens *et al.*, 1997c). No transformations were applied to the data to prevent errors during the estimation (Ross, 1990). The data were analysed as one integral set using time and temperature simultaneously as explaining variables (Tijskens, 1994).

Most of the experiments are conducted at constant conditions of external factors like for example temperature. To analyse the experimental data analytical solutions of the model formulation at constant external conditions is required. These analytical solutions will be deduced from the differential equations, but are only applicable at constant conditions. In practice constant conditions are very rare. However, the model formulations applicable at any time and temperature are the differential equations. The formulation of the differential equations is the core of the model rather than the resulting analytical solutions. These analytical solutions are a logical consequence of the differential equations. The boundary conditions for the differential equations are defined by the experimental set-up.

### **Symbols and notation used**

Within the notation used to describe the underlying chemical and biochemical processes affecting the firmness of agro-products the following has to be realised. With reference to the enzymes, the activities of these enzymes were determined *in vitro* under standardised conditions with respect to the amount of substrate, pH, buffer, temperature etc. These enzymes were extracted from the agro-products under study, which were either stored or heat treated under defined conditions. So the increase or decrease in activity was related to either formation of enzyme or inactivation of enzyme. Since all the modelling work was based on the information supplied by the *in vitro* assays, these activities served as input information for the models and not the enzyme amount, or concentration. For this reason in the modelling part the notation referring to enzymes was not put in between brackets to distinguish it from real concentrations.

As a consequence of enzymatic reactions pectin is either demethylated (PE action), or depolymerised enzymatically (PG action), or chemically ( $\beta$ -degradation). Within the study presented neither the DE, nor the DP of pectin was determined. What is mathematically described are the consequences of either chemical, or enzymatic breakdown of the pectic polymer and these effects are related to changes in firmness.

Since neither the DP, nor the DE are concentrations, but rather relative amounts, these notations were not put in between brackets either.

## **Modelling approach**

### **The mode of action of the enzyme activities to be modelled**

In this chapter three enzymes are discussed. Their function and activity are assumed to be (strongly) related to the texture of fruits and vegetables. Two of these enzymes have pectin as their main substrate. These enzymes are respectively pectin methyl esterase (PE; EC 3.1.1.11) and endo-polygalacturonase (PG; EC 3.2.1.15). PE removes methanol from methylated pectin. The plant enzyme works as a zipper and removes the methyl groups blockwise, in contrast to the fungal enzyme which demethylates pectin's more at random (Pilnik & Voragen, 1991; Burns, 1991). The consequences of the PE action are threefold. Firstly, due to its blockwise demethylating action the enzyme increases the probability that two adjacent polygalacturonic polymer chains form "egg-boxes" in the presence of calcium ions. The formation of a three dimensional network, also caused by "egg-box" structures, results in an apparent increase in the chain length of the pectic polymers. As a consequence, the firmness of the plant tissue is increased. In addition, this demethylation process



enhances both shielding and repulsion forces by the electric charges within the pectic polymer matrix of the cell wall and middle lamellae.

Secondly, demethylated pectin is, in contrast to methylated pectin, not vulnerable to  $\beta$ -eliminative breakdown (Van Buren & Peck, 1981; Sajjaanantakul *et al* 1989; Keybets & Pilnik, 1974).  $\beta$ -Eliminative breakdown is virtually absent at room temperature: its rate constant  $k \approx 0$ . It starts to contribute to observable changes in firmness above about 90 °C. Therefore, it is assumed that preheating of plant tissue at moderate temperatures (50 – 80 °C) reduces the softening of the plant tissue compared to no preheating treatment.

Thirdly, demethylated pectin forms the substrate for PG. Due to its mode of action, PG action is assumed to decrease firmness, since this enzyme depolymerises the pectin, thereby decreasing its DP.

The second enzyme studied is PG. This enzyme is observed in many fresh fruits and vegetables (Pilnik & Voragen, 1991). It depolymerises pectic polysaccharide chains preferably at those locations where the methyl groups have been removed. Due to its mode of action the DP of the pectic polysaccharide chains decreases. This decrease in DP is assumed to be, at least (partly) responsible for the frequently observed softening of plant tissue (Shewfelt, 1965; Shewfelt *et al.*, 1971). However, conflicting information exists about the observed PG activity and fruit softening (Awad & Young, 1979; Grierson & Frey, 1994; Christopher *et al.* 1990; Giovanni *et al.* 1989, Smith *et al.* 1988).

The third enzyme studied is peroxidase (POD; EC 1.11.1.7). POD is one of the plants most heat resistant enzymes and is therefore often used as marker enzyme to assess the effectiveness of a heat treatment (Robinson, 1995) POD is also assumed to be involved in the oxidative cross-linking of cell wall polymers by catalysing the formation of for example diferulic acid and isodityrosine crosslinks (Parker & Waldron, 1995; Parr *et al*, 1996; Brett, & Waldron, 1990; Fry, 1988).

Two remarks are at place. With regard to the enzymes mentioned it has to be realised that these enzymes just form a small part of all enzymes involved in cell wall metabolism. However, these enzymes are assumed to have the highest contribution to enzyme catalysed, texture-modifying actions. The second remark refers to the texture of plant materials. During either storage or heat processing the firmness of the plant material decreases to a certain minimal value. In other words the firmness of plant material consists of two components, a fixed part ( $Firm_{fix}$ ) and a variable part ( $Firm_{var}$ ). The firmness generated by the cellulose-hemicellulose domain of the plant cell wall will hardly be affected during heat processing (Hinton & Pressey, 1973), let alone during storage and thus represents the fixed part of the firmness. The variable part of the firmness refers to the pectic fraction of the cell wall. Its chemical composition, and as a consequence thereof its physical properties, are affected by either the enzymes such as described above or enhanced temperatures.

### ***Firmness decrease during storage, with peaches as example***

In Figure 11-1 the measured PG activity of peaches harvested at two sequential years is shown (Tijsskens *et al.*, 1998). The first observation is that the form of the curves at each storage temperature used are similar; at increasing storage temperatures the PG activity first increases in time followed by a decrease in activity. The second observation is that the total observed activity differs with a factor of about twenty between years. The observed increase in PG activity during the early stages of storage and the observed decrease in activity upon prolonged storage, can be explained with the following mechanism. An increase in enzyme activity is due to the formation of active enzyme from an inactive, latent form of the enzyme ; a decrease in activity can be caused by inactivation of the enzyme. At storage temperatures it is difficult to imagine that enzyme denaturation occurs; therefore (senescence) inactivation is assumed. This behaviour of PG can be described as a set of consecutive reactions (see eqns. 1a and 1b).





This set of chemical reactions can be converted into a set of differential equations (eqns. 2a and 2b) and solved analytically for constant temperatures (eqn. 3).

$$\frac{\partial PG_{pre}}{\partial t} = -k_f \cdot PG_{pre} \quad (2a)$$

$$\frac{\partial PG}{\partial t} = k_f \cdot PG_{pre} - k_d \cdot PG \quad (2b)$$

$$PG(t) = PG_{pre,0} \cdot k_f \cdot \left( \frac{e^{-k_d \cdot t} - e^{-k_f \cdot t}}{k_f - k_d} \right) + PG_0 \cdot e^{-k_d \cdot t} \quad (3)$$

Eqn. 3 describes the total PG activity at any time at a given temperature. In these equations  $t$  is the time of storage, PG is the activity of the enzyme measured under standard conditions,  $k$  is the reaction rate constant. The index "pre" refers to the inactive precursor, "f" refers to the formation process of active enzyme, "d" refers to the denaturation process, "na" refers to the inactivated enzyme, and "0" refers to the initial condition ( $t=0$ ; with reference to the start of the measurements). The temperature dependence of chemical reactions is described by Arrhenius' law. Therefore both reaction rate constants  $k_f$ , and  $k_d$  (see eqn. 1) are assumed to depend on temperature according this law (see eqn. 4).

$$k_i = k_{i,ref} \cdot e^{\frac{E_{a_i}}{R} \cdot \left( \frac{1}{T_{ref}} - \frac{1}{T_{abs}} \right)} \quad (4)$$

In this equation  $T_{ref}$  refers to a chosen reference temperature (K),  $T_{abs}$  is the storage temperature (K),  $k_{i,ref}$  is the reaction rate constant "i" of a chemical reaction at  $T_{ref}$ .  $E_{a_i}$  is the energy of activation and  $R$  is the universal gas constant.

The above series of differential equations describe the observed phenomena as depicted in Figure 11-1, and their relation with temperature dependence of the underlying rate constants according to Arrhenius'.

The data on the PG activities, measured in peaches stored at different constant temperatures, were analysed statistically using eqn. 3 for the time dependence and eqn. 4 for the temperature dependence of all reactions and their rate constants involved (see eqns. 1 and 2). The results of this statistical analysis for the two seasons studied

Table 11-1 Results of the statistical analysis for the PG activity measured in stored peaches during two seasons and of the combined analysis

<i>Parameter</i>	<i>Season 1</i>	<i>Season 2</i>	<i>Season (1+2)</i>
	<i>Estimate</i>	<i>Estimate</i>	<i>Estimate</i>
<i>Kinetic parameters</i>			
$k_{f,ref}$	$1.73 \cdot 10^{-2}$	$6.64 \cdot 10^{-2}$	$6.38 \cdot 10^{-1}$
$E_{a_f}/R$	$1.36 \cdot 10^4$	$6.27 \cdot 10^3$	$1.17 \cdot 10^4$
$k_{d,ref}$	$1.79 \cdot 10^{-1}$	$2.69 \cdot 10^{-1}$	$1.02 \cdot 10^{-1}$
$E_{a_d}/R$	$5.67 \cdot 10^3$	0 (fixed)	0 (fixed)
<i>Batch parameters</i>			
$PG_{0,season 1}$	$3.00 \cdot 10^{-1}$	n.a.	$2.35 \cdot 10^{-1}$
$PG_{pre,0,season 1}$	8.37	n.a.	2.28
$PG_{0,season 2}$	n.a.	$1.25 \cdot 10^1$	$1.82 \cdot 10^1$
$PG_{pre,0,season 2}$	n.a.	$1.22 \cdot 10^2$	$4.09 \cdot 10^1$
<i>Administrative information</i>			
$T_{ref}$	10	10	10
$R^2_{adj}$	81.8	85.2	81.6
$N_{obs}$	31	30	61
n.a.	not applicable		

independently and for the combined information of the two seasons together is given in Table 11-1.

Based on the results shown in Table 11-1, the following conclusions can be made. Firstly, the model is capable of describing the apparent different PG activity between seasons (see

Figure 11-1) with the same estimates for the kinetic parameters. This strongly suggests, that the classification of the parameters used for the kinetic parameters (cultivar specific) and batch parameters (batch/season specific) is allowed and valid. Secondly, it also proves that the model formulation, in conjunction with the assumptions made, is valid, irrespective of growth and harvest conditions. In other words, the model is capable of dealing with stochastic behaviour during successive seasons and growing areas.

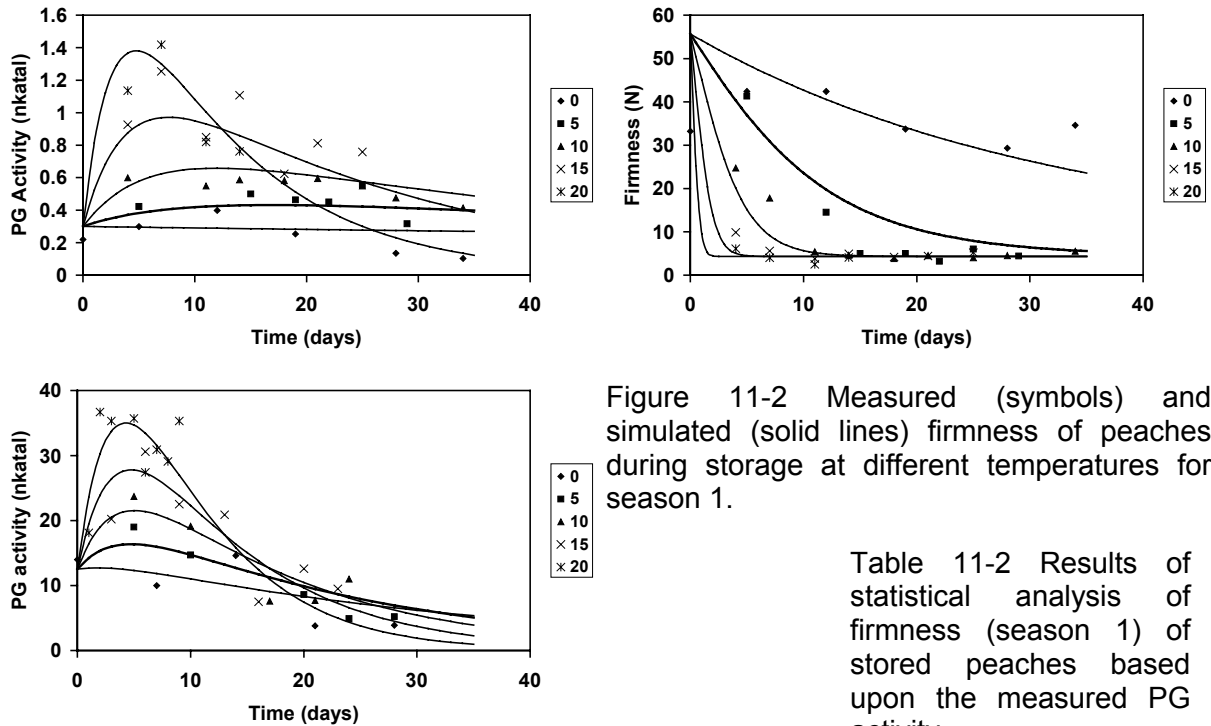


Figure 11-1 Measured (symbols) and simulated (solid lines) PG activities of peaches during storage at different temperatures for season 1 (top) and season 2 (bottom).

The next question to be answered is of course how this information, contained in a set of differential equations, can be related to the observed changes in firmness (see Figure 11-2). So, the basic activity of PG, decreasing the DP of the pectin matrix, has also to be described in differential equations. The peach variety studied for the storage experiments (cv. Red Haven) contained virtually no PE activity. If PE would be present in time increasing amounts of pectin would be demethylated which results in an increase in the amount of substrate for PG. Since PE is absent this reaction does not have to be included into the model formulation and DE can be considered constant throughout the storage period. The amount of active PG affects the DP of pectin. This can be described according to the following mechanism and differential equation:



$$\frac{\partial DP}{\partial t} = -k_{PG} \cdot PG \cdot DP \quad (6)$$

In these equations PG is the activity of polygalacturonase, DP is the degree of polymerisation of pectin, and  $k_{PG}$  is the reaction rate constant of the depolymerisation reaction. The chemical reaction (eqn. 5) describes that PG depolymerises PG accessible

Figure 11-2 Measured (symbols) and simulated (solid lines) firmness of peaches during storage at different temperatures for season 1.

Table 11-2 Results of statistical analysis of firmness (season 1) of stored peaches based upon the measured PG activity

Parameter	Estimate
<i>Kinetic parameters</i>	
$k_{PG,ref}$	$6.70 \cdot 10^{-1}$
$Ea_{PG/R}$	$1.48 \cdot 10^4$
<i>Batch parameters</i>	
$Firm_{var}$	$5.13 \cdot 10^1$
$Firm_{fix}$	4.32
<i>Administrative information</i>	
$T_{ref}$	10
$R_{adj}^2$	89.2
$N_{obs}$	32

pectin and that pectin becomes depolymerized without changing the amount of active enzyme. Eqn. 5 can be expressed in its differential equation (eqn. 6) by again applying the fundamental rules of chemical kinetics. Substituting the analytical solution for PG (eqn. 3) into this differential equation and solving for constant conditions (constant temperatures), one obtains eqn. 7.

$$\frac{DP(t)}{DP_0} = e^{k_{PG} \cdot PG_0 \cdot \left( \frac{e^{k_d \cdot t} - 1}{k_d} \right) - k_{PG} \cdot PG_{pre,0} \cdot \left( \frac{k_d - k_f + k_f \cdot e^{-k_d \cdot t} - k_d \cdot e^{-k_f \cdot t}}{k_d \cdot (k_d - k_f)} \right)} \quad (7)$$

Eqn. 7 describes the degree of polymerisation at a given time, at constant temperature conditions, due to the action of PG. As indicated earlier, the firmness of plant products consists of a variable part (pectin based) and a fixed part (cellulose-hemicellulose based). Since all changes in firmness are ascribed to changes in the DP of pectin, eqn. 7 is accordingly converted into an equation describing the changes in observed firmness on basis of the enzyme catalysed depolymerisation of the pectin matrix:

$$\text{Firm} = \text{Firm}_{\text{fix}} + \text{Firm}_{\text{var}} \cdot \frac{DP(t)}{DP_0} \quad (8)$$

The firmness data (see Figure 11-2) of stored peaches (season 1) were analysed using eqn. 8 and eqn. 4 simultaneously. The values of the kinetic parameters  $k_{f,ref}$ ,  $E_a/R$ , and the batch parameters  $PG_0$  and  $PG_{pre,0}$  were used such as determined (see Table 11-1, season 1). The results of this analysis is given in Table 11-2.

The fact that the firmness behaviour can be explained by the activity of the appropriate enzyme, estimated on separate enzyme data, assessed *in vitro*, with a  $R^2_{adj}$  of about 90% strongly indicates and confirms the reliability of the modelling approach used.

The simulated data using the estimated parameters of Table 11-1 and Table 11-2 are represented by the solid lines in Figure 11-2.

### **The activity of pectin methyl esterase during mild heat treatments of potatoes, carrots and peaches**

The main aims of a heat treatment of agro-products are twofold. First of all a heat treatment decreases the microbial load of these products, thereby extending their keeping quality or shelf life. The second aim is the activation and/or inactivation of enzymes present in the plant tissue (Pilnik & Voragen, 1991). The apparent activity of enzymes exhibits a well known behaviour. At temperatures below about 50 °C a continuous increase in activity is observed with increasing temperatures. This increase in activity can generally be described by Arrhenius' law. At still higher temperatures a rather steep decline in the apparent activity is observed due to denaturation. (Segel, 1993; Whitaker, 1994; Wiley, 1994).

One of the enzymes presumed to have an effect on the firmness of heat treated plant products is the enzyme pectin methyl esterase (PE). As such, PE has a long record of confusing effects on the contribution to firmness during heat treatments. Obviously the relation between measured PE activity and observed firmness is complex. One of the factors, which might add to this confusion, is the existence of different PE -isoenzymes (Recourt *et al.*, 1996; Ebbelaar *et al.*, 1996) and the observation that PE can either be soluble or bound to the cell wall polysaccharides (Laats *et al.*, 1997).

In order to obtain a better insight in the effect of mild heat treatments for two vegetables (carrots and potatoes) and one fruit (peaches) the effect of a range of temperatures was used to determine the effect at a given temperature on the observed PE activities in time. A mathematical model was developed that describes the dynamic changes in PE activity. For more detailed information on the vegetables see (Tijsskens *et al.* 1997b) and on the peaches see (Tijsskens *et al.* 1999).

In Figure 11-3 the measured PE activities for carrots, potatoes and peaches as function of heating time and temperature are shown respectively. For carrots and potatoes (Figure 11-3 Top left & Top right) up to 70 °C an (initial) increase in the measured PE activity can be

observed. Obviously below this temperature additional PE activity is formed. Above this temperature the decrease in activity seems to be exponential, indicative of a first order inactivation process. The formation process is apparently completely overruled by the inactivation process at temperatures higher than 70 °C. For peaches a different behaviour is observed. At temperatures below 70 °C, in contrast to carrots and potatoes, no (initial) increase in measured PE activity can be observed; a heat resistant PE activity seems to exist. An exponential decrease in activity in time seems only to occur at temperatures above 70 °C. To account for these observations in the three products studied a conversion from a bound to the soluble PE activity is assumed to occur. This assumed behaviour can be represented in a set of chemical reactions given in eqn. 9.



The total activity of the PE enzyme comprises both the bound and the soluble configuration:

$$PE_{\text{tot}} = PE_{\text{bnd}} + PE_{\text{sol}} \quad (10)$$

The index "sol" indicated the soluble fraction, "bnd" the bound fraction, "c" the conversion reaction and "d" the denaturation of the enzyme. This set of chemical reactions can be converted by fundamental kinetics into a set of differential equations:

$$\frac{\partial PE_{\text{bnd}}}{\partial t} = -k_{d,\text{bnd}} \cdot PE_{\text{bnd}} - k_c \cdot PE_{\text{bnd}} \quad (11a)$$

$$\frac{\partial PE_{\text{sol}}}{\partial t} = k_c \cdot PE_{\text{bnd}} - k_{d,\text{sol}} \cdot PE_{\text{sol}} \quad (11b)$$

At constant temperatures, the solution of this set of differential equations for the sum of both active configurations of the enzyme ( $PE_{\text{tot}}$ ) is given in eqn. 12a.

$$PE_{\text{tot}} = PE_{\text{sol},0} \cdot \exp(-k_{d,\text{sol}} \cdot t) + PE_{\text{bnd},0} \cdot \left( \frac{(k_{d,\text{bnd}} - k_{d,\text{sol}}) \cdot \exp\{- (k_c + k_{d,\text{sol}}) \cdot t\} + k_c \cdot \exp(-k_{d,\text{sol}} \cdot t)}{k_{d,\text{bnd}} + k_c - k_{d,\text{sol}}} \right) \quad (12a)$$

In case the conversion of bound enzyme to far precede its denaturation (the reaction rate for the inactivation process,  $k_{d,\text{bnd}}$  is much smaller than that of the formation process,  $k_c$ ), as is the case for carrots and potatoes, eqn. 9b is not of relevance. As a consequence eqn. 12a simplifies to:

$$PE_{\text{tot}} = PE_{\text{sol},0} \cdot \exp(-k_{d,\text{sol}} \cdot t) + PE_{\text{bnd},0} \cdot \left( \frac{k_c \cdot \exp(-k_{d,\text{sol}} \cdot t) - k_{d,\text{sol}} \cdot \exp(-k_c \cdot t)}{k_c - k_{d,\text{sol}}} \right) \quad (12b)$$

The first term in eqns. 12a and 12b describe the denaturation of the initially present soluble PE activity. The second term describes the unbinding of PE and the denaturation of the enzyme in its unbound form (eqn. 12b; carrots and potatoes) or denaturation of both its bound and its unbound form (eqn. 12a; peaches).

Again, by applying Arrhenius' law to the reaction rate constants in combination with the equations for denaturation (eqns. 12a and b), the general pattern of enzyme denaturation at any constant temperature can be described. The data were analysed with eqn. 12a for peaches and eqn. 12b for carrots and potatoes together with eqn. 4 (Arrhenius' law) using non-linear regression, with time and temperature simultaneously as dependent variables.

For the three products analysed the initial level of PE activity was slightly different for each temperature-time activity curve (see Figure 11-3).

Table 11-3 Results of the statistical analysis for the PE activity of carrots, potatoes and peaches (two seasons combined)

Parameter	Estimate for		
	Carrots	Potatoes	Peaches
<i>Kinetic parameters</i>			
$k_{c,ref}$	2.35	$9.42 \cdot 10^{-2}$	$1.03 \cdot 10^{-2}$
$Ea_c/R$	$1.40 \cdot 10^4$	$1.30 \cdot 10^4$	$8.03 \cdot 10^3$
$k_{d,bnd,ref}$	n.a.	n.a.	$8.00 \cdot 10^{-3}$
$Ea_{d,bnd}/R$	n.a.	n.a.	$5.00 \cdot 10^2$
$k_{d,sol,ref}$	$4.25 \cdot 10^{-2}$	$6.18 \cdot 10^{-2}$	$3.91 \cdot 10^{-4}$
$Ea_{d,sol}/R$	$1.96 \cdot 10^4$	$1.72 \cdot 10^4$	$6.81 \cdot 10^4$
<i>Batch parameters</i>			
PE <sub>sol</sub> ; Activity range	9.01 - 10.0	3.17 - 6.29	n.a.
PE <sub>bnd,0</sub>	1.32	2.48	n.a.
PE <sub>bnd</sub> ; Activity range	n.a.	n.a.	0.84 - 10.8
PE <sub>sol,0</sub>	n.a.	n.a.	4.88
<i>Administrative information</i>			
R <sup>2</sup> <sub>adj</sub>	89.1	84.4	91.5
T <sub>ref</sub>	60	60	60
N <sub>obs</sub>	159	160	90
n.a.;	not applicable		

This can be caused by either differences between batches and/or experimental errors. For this reason the initial PE activity levels were estimated separately for each temperature allowing each temperature series to have its own initial value; the kinetic parameters were estimated in common. The results of this analysis for the individual data for carrots, potatoes and peaches (results of two seasons combined) are given in Table 11-3.

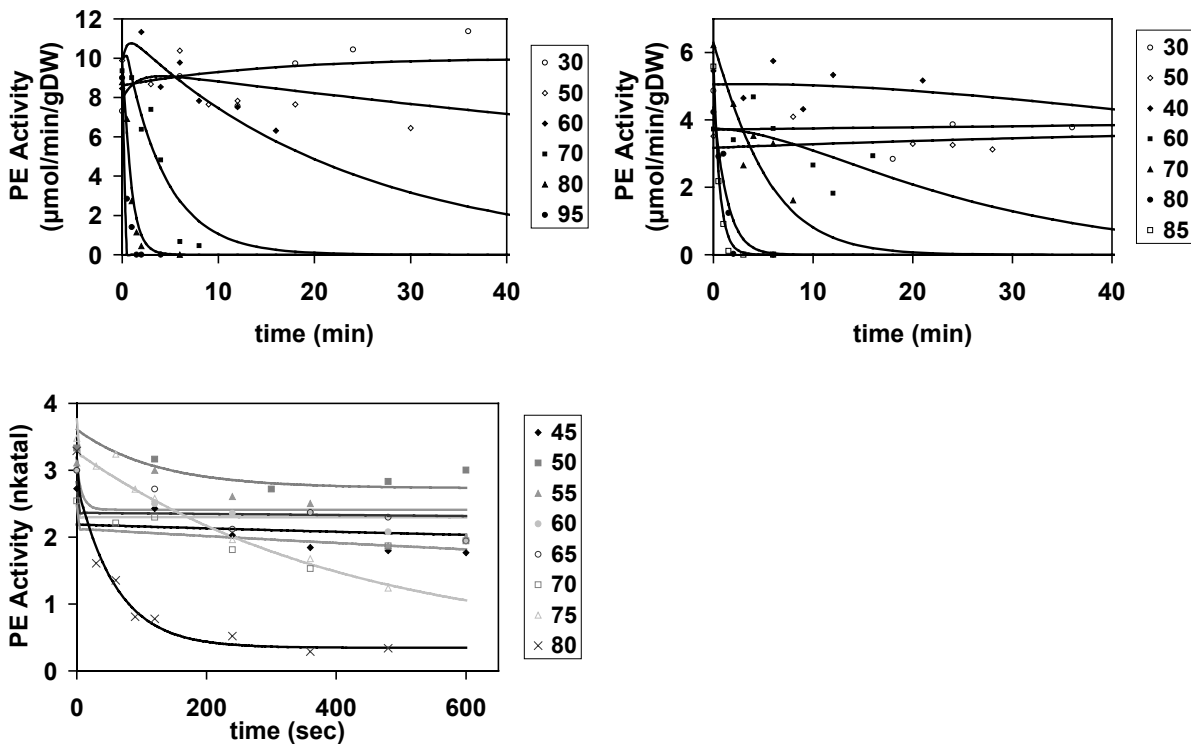


Figure 11-3 Mean (symbols) and simulated (solid lines) of PE activity as function of time and preheating temperature. Top left: carrots, Top right: potatoes, Bottom: peaches.

The results shown for peaches (Figure 11-3 C) suggest two active configurations of the PE; the bound form ( $PE_{\text{bnd}}$ ) prevailing at the lower temperature region, the soluble form ( $PE_{\text{sol}}$ ) prevailing at the higher temperatures by a rapid temperature dependent conversion (see eqn 11a). The reaction rate constant of the denaturation at the reference temperature for the soluble PE form ( $k_{\text{d,s,ref}}$ ) is smaller than for the bound PE configuration ( $k_{\text{d,b,ref}}$ ), which indicates a better heat stability at the reference temperature for the soluble PE. The activation energy for the denaturation of the bound configurations ( $E_{\text{d,bnd}}$ ) is much smaller than for the soluble form ( $E_{\text{d,sol}}$ ). The consequence of this difference is that the denaturation of the soluble configuration is more affected by a temperature increase than the denaturation of the bound form.

The fact that both the solubilisation reaction and the two inactivation reactions (see eqn. 11) are required to explain the observed behaviour, signifies that both reactions can precede the other at some situation in time and/or temperature. In Figure 11-4 the three dimensional simulation of the PE activity of carrots (top) and peaches (bottom) as function of time and (constant) temperature are shown respectively, based on the results of analysis (Table 11-3).

The striking difference of this simulation between carrots and potatoes (the latter is not shown, since it is almost similar as for carrots) at one hand, and peaches at the other hand is that the PE of carrots and potatoes almost obey a first order inactivation mechanism and that the inactivation of PE of peaches occurs in two sequential steps.

For peaches the explained parts,  $R^2_{\text{adj}}$ , for the first and second season analysed independently are respectively 93% and 90%. For the analysis of the combined information this value is 91.5% showing no loss of explaining power. The major advantage of combined analysis lies in the increase in explaining and predicting power for different situations and different batches. Furthermore the fact that the activity of PE in peaches of different seasons, and therefore different properties, can be analysed together with the same model, and the most important parameters in common, constitutes a major validation of the principles, assumptions and deduction techniques underlying this model.

In Figure 11-4 the three dimensional simulation of the PE activity of carrots and peaches as a function of time and temperature is shown respectively. For carrots it is obvious that the enzyme activity decays (almost) exponentially in time at any temperature.

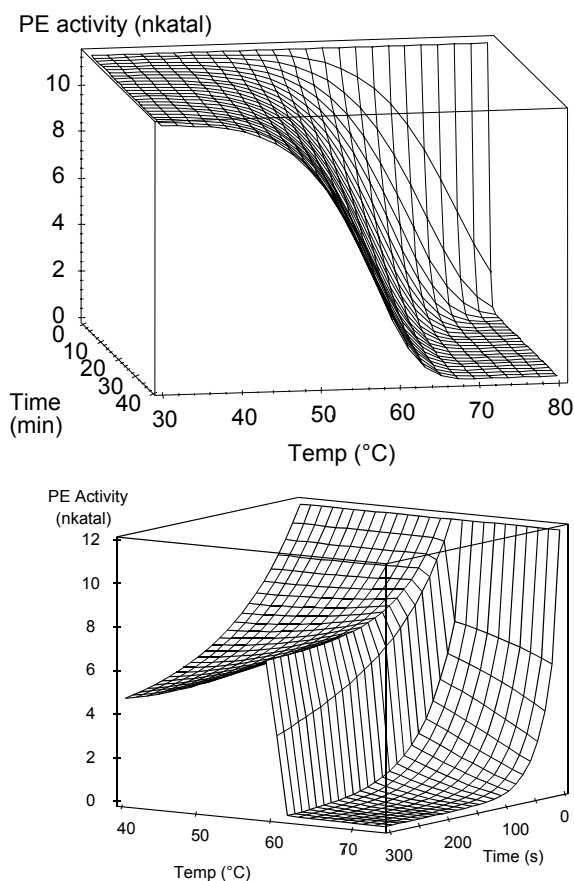


Figure 11-4 Three dimensional simulation of the PE activity as function of time and temperature, based on the parameter estimates of Table 11-3. Top: in carrots, Bottom: peaches

### ***The activity of peroxidase during mild heat treatments of potatoes, carrots and peaches***

The enzyme peroxidase (POD) is found in almost all living organisms. The primary action of this enzyme is to control the level of peroxides, generated in oxygenation reactions, to avoid excessive formation of radicals, being harmful to all living organisms. Due to its relatively high heat stability POD serves in the processing of fruits and vegetables as marker enzyme

(Robinson, 1995). It is assumed that if a tissue is peroxidase negative, that all enzymes are denatured. With regard to texture this enzyme regained attention due to its potential to establish phenolic cross-links between either neighbouring polymers in relation to cell-cell adhesion and the consequences for thermal stability of texture (Parker & Waldron, 1995; Parr et al. 1996). To gain insight in the temperature-time of this enzyme, as was the case for PE (discussed earlier), two vegetables (carrots and potatoes) and one fruit (peaches) were studied (Tijssens et al. 1997a). The plant enzyme consists of a complex spectrum of isoenzymes (Shannone, 1968). Furthermore the existence of both soluble and bound isoenzymes has been reported each with different susceptibilities to heat denaturation (Gkinis & Fenema, 1978). In other words, from the enzymatically active bound form of POD the enzymatically active soluble form is generated, both catalytically active forms being susceptible to heat denaturation. Comprising this information into a mathematical formulation leads to:



describing the solubilisation of the bound POD



and eqns. 13b,c describing the thermal denaturation process of both the bound and soluble enzyme. This reaction mechanism is the same as described for the PE activity in peaches (see earlier and Tijssens *et al.*, 1999).

The total activity of the POD enzyme comprises both the bound and the soluble enzyme configurations:

$$\text{POD}_{\text{tot}} = \text{POD}_{\text{bnd}} + \text{POD}_{\text{sol}} \quad (14)$$

This set of chemical reactions can be converted into a set of differential equations:

$$\frac{\partial \text{POD}_{\text{bnd}}}{\partial t} = -k_{d,\text{bnd}} \cdot \text{POD}_{\text{bnd}} - k_c \cdot \text{POD}_{\text{bnd}} \quad (15a)$$

$$\frac{\partial \text{POD}_{\text{sol}}}{\partial t} = -k_{d,\text{sol}} \cdot \text{POD}_{\text{sol}} + k_c \cdot \text{POD}_{\text{bnd}} \quad (15b)$$

As can be observed in Figure 11-5, at prolonged times and elevated temperatures still POD activity is observed for both the bound as well as the soluble form. This fixed part of the activities is of course not included in these differential equations, but is accounted for in the

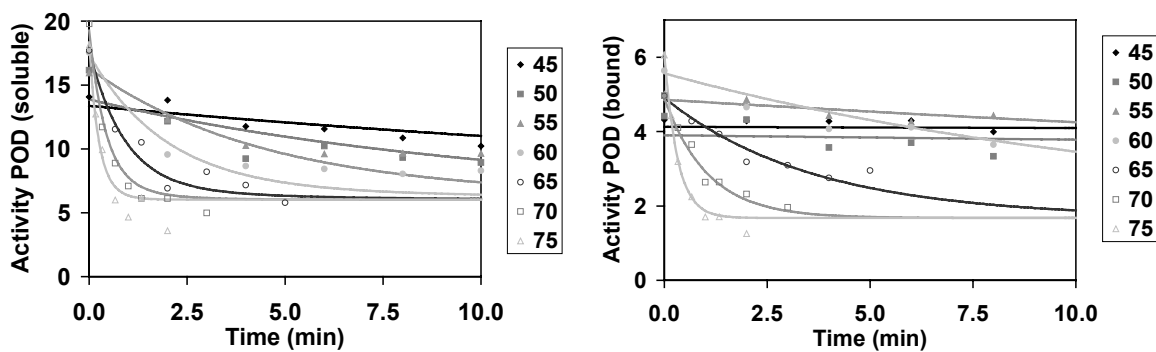


Figure 11-5 Measured (symbols) and simulated (solid lines) activity of POD activity in peaches as function of preheating temperature and time. Left: Soluble POD, Right: bound POD.



analytical solutions of eqn. 16.

The analytical solution of this set of differential equations for the sum of both the bound and soluble form of this enzyme is, taking the fixed part into consideration results in eqn. (16).

$$POD_{bnd} = POD_{bnd,var} \cdot e^{-(k_{d,bnd}+k_c) \cdot t} + POD_{bnd,fix} \quad (16a)$$

$$POD_{sol} = POD_{sol,var} \cdot e^{-k_{d,sol} \cdot t} + POD_{sol,fix} + POD_{bnd,var} \cdot \left( \frac{k_c \cdot (e^{-k_{d,sol} \cdot t} - e^{-(k_{d,bnd}+k_c) \cdot t})}{k_{d,bnd} + k_c - k_{d,sol}} \right) \quad (16b)$$

The first term in eqn. 16a describes the denaturation of the initially present bound POD activity. The second term describes the invariable part of the bound activity for the observed activity remaining after heat treatment. In eqn. 16b the first term describes the denaturation of the initially present soluble POD activity. The second term describes an invariable part of the soluble POD activity for the observed activity remaining after heat treatment. The third term describes the combination of heat denaturation of the bound POD activity and the formation of the soluble POD activity from the bound POD activity. By applying Arrhenius' law (eqn. 4) to the reaction rate constants in combination with the equations for denaturation (eqns 16), the general pattern of enzyme denaturation at any constant temperature can be described.

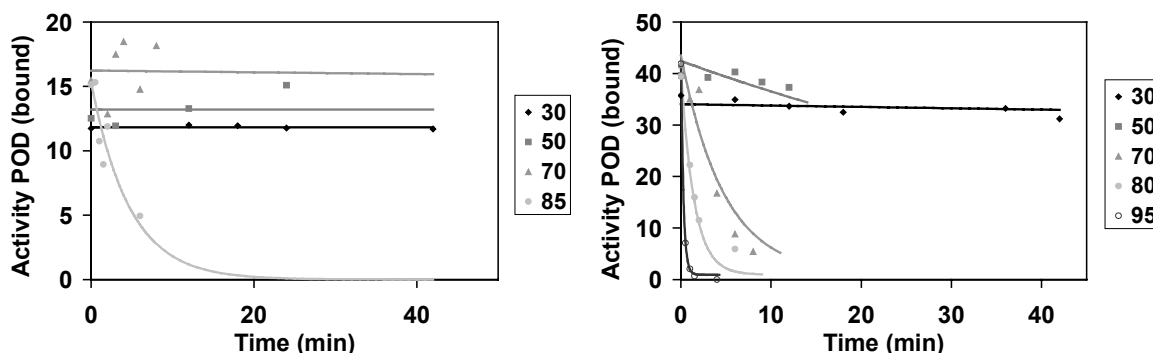


Figure 11-6 Measured (symbols) and simulated (solid lines) activity of bound POD activity as function of preheating temperature and time. Left: Potatoes, Right: carrots

For carrots and potatoes only the bound enzyme was assayed, for peaches both the soluble and bound enzyme was assessed separately. Consequently, the analysis of the enzyme data on peaches is more elaborate but also more comprehensive and reliable than for potatoes and carrots. In Figure 11-5 both the soluble (Figure 11-5 A) and bound activity (Figure 11-5 B) of POD in peaches as function of heating time and temperature is shown. In Figure 11-6 the bound POD activity as function of heating time and temperature is shown for carrots (Figure 11-6 A) and potatoes (Figure 11-6 B). As with the PE activity in peaches (Tijskens *et al.* 1999), carrots and potatoes (Tijskens *et al.* 1997b) the initial POD activity was slightly variable on a day-to-day basis. Therefore the initial POD activity levels (bound and soluble) were estimated separately, the kinetic parameters were estimated in common (see earlier).

During data analysis  $k_{dbnd,ref}$  consistently approached zero. So, the denaturation of the bound form is not supported by the data. Apparently, the conversion of the bound form into the soluble configuration is faster and more important than the denaturation of the bound configuration. In other words, all the heat labile bound POD will already be solubilized by the time direct denaturation of the bound POD occurs. During further statistical analysis of the data  $k_{dbnd,ref}$  was fixed at zero. A compilation of the of the estimated values for peaches, carrots and potatoes is given in Table 11-4.

The explained part,  $R^2_{adj}$ , for both carrots and peaches is about 95% indicating the

correctness of the assumptions being made. For potatoes the explained part is lower (70%). This could be caused by the limited number of objects studied.

The results of the statistical analysis strongly indicate that the bound form of POD solubilizes and is subsequently denatured. The heat labile bound POD will already be solubilized by the time direct inactivation of the bound POD occurs.

The simulated three-dimensional activity-time-temperature behaviour of the POD activity in peaches, both bound and soluble, is shown in Figure 11-7. The values of the parameters to perform this simulation were from Table 11-4, the value for  $POD_{sol,var}$  was set at 10.6, the value for  $POD_{bnd,var}$  was set at 3.23. Viewed along the time axis, the POD activity, both bound and soluble configurations, behave roughly according an exponential decay process at constant temperature. Viewed along the temperature axis, the difference in susceptibility to disappear either by conversion or denaturation can be observed.

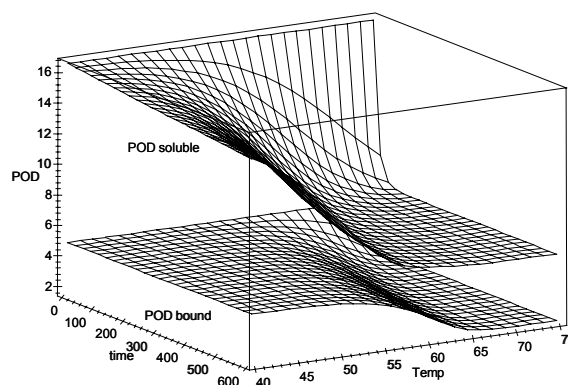


Figure 11-7 Three dimensional of bound and soluble POD activity of peaches as function of time and temperature, based on the parameter estimates of Table 11-4.

### A Preheating -Cooking Model:

#### BRAM: Blanching Response Amplification Model

In the previous sections, attention has been given to the formulation of the kinetic reactions assumed to underlie the observed enzyme activity and their time-temperature dependent behaviour in the form of differential equations and analytical solutions. By proper merging of these analytical solutions with Arrhenius' law, estimates were made about the rate constants and activation energies. For stored peaches, the PG activity was directly related to the change in firmness of this fruit.

Preheating is, as mentioned earlier, one of the means to decrease the microbial load of agro-products thereby extending their keeping quality or shelf life. However, preheating as such is also assumed to have an effect on the firmness of the cooked or sterilised products. In this section the effect of preheating on the firmness of the cooked products will be formulated in a set of reaction mechanisms. These mechanisms will be developed into mathematical equations according to the strategy as discussed in the previous sections. Emphasis is focused on the observed firming effect of PE during preheating and the consequences for the texture of the cooked product. Here it has to be realised that the total process is

Table 11-4 Results of the statistical analysis for the POD activity of carrots, potatoes and peaches

Parameter	Estimate for		
	Carrots	Potatoes	Peaches
<i>Kinetic parameters</i>			
$k_{c,ref}$	$9.61 \cdot 10^{-4}$	$8 \cdot 10^{-7}$	$1.30 \cdot 10^{-3}$
$Ea_c/R$	$1.42 \cdot 10^4$	$5.10 \cdot 10^4$	$2.85 \cdot 10^4$
$k_{d,bnd,ref}$	n.a.	n.a.	0 (fixed)
$Ea_{d,bnd}/R$	n.a.	n.a.	0 (fixed)
$k_{d,sol,ref}$	n.a.	n.a.	$8.44 \cdot 10^{-3}$
$Ea_{d,sol}/R$	n.a.	n.a.	$1.80 \cdot 10^4$
<i>Batch parameters</i>			
$POD_{bnd,fix}$	0 (fixed)	0 (fixed)	1.68
$POD_{bnd,var}$ :activity range	34.1 - 43.4	0.53-1.10	2.22 - 4.44
$POD_{sol,fix}$	n.a.	n.a.	6.03
$POD_{sol,var}$ :activity range	n.a.	n.a.	7.34 - 12.3
<i>Administrative information</i>			
$R^2_{adj}$	94.5	70.0	96.4
$T_{ref}$	60	60	60
$N_{obs}$	216	21	90
n.a.	not applicable		

divided into two sub-processes, preheating and cooking.

During preheating PE exerts its action, demethylation of pectin. The preheating is performed at temperatures below 90 °C. Virtually no  $\beta$ -degradation of pectin takes place below this temperature. It has however to be emphasised again that the amount of sites vulnerable to  $\beta$ -degradation decreases due to the PE action.

During cooking all PE is inactivated very fast (a  $10^3$ -fold decrease in activity within 20 sec.) so the cooking process does virtually not change the degree of methylation any more. However, the sites not affected by the PE action are vulnerable to  $\beta$ -degradation during cooking. In Figure 11-4 A the three-dimensional comprised of the PE activity of carrots is shown as function of (preheating) time and temperature, based on the results of the statistical analysis of the PE activity (see Table 11-3). The bound form of PE ( $PE_{\text{bound}}$ ) of carrots comprises only about 10% of the total PE activity, as defined in eqn.11. To simplify both the mathematics and the statistical analysis the bound form was not included in the analysis to simplify the system and to make a statistical analysis possible.

To distinguish between the preheating and the cooking time the indices “p” and “c” are respectively used to indicate the different types of processing.

For the preheating process to develop the preheating-cooking model, the following reactions and time dependent equations are formulated:

*Preheating:*



$$\frac{\partial PE}{\partial t_p} = -k_d \cdot PE \quad (17b)$$

In eqn. 17 the bound form of PE was neglected. The reaction of methylated pectin with PE is described with eqn. 18.



$$\frac{\partial DE}{\partial t_p} = -k_s \cdot DE \cdot PE \quad (18b)$$

This reaction states that the PE accessible pectin is demethylated in time without changing the amount of active enzyme. However, the preheating process leaves the demethylated pectin backbone with the same DP as before the action of PE.

Substituting eqn. 17 into eqn. 18 results in:

$$DE(t_p) = DE_0 \cdot e^{-\frac{k_s \cdot PE_0 \cdot (e^{-k_d \cdot t_p} - 1)}{k_d}} \quad (19)$$

Eqn. 19 describes the DE at any time during the preheating process as a consequence of the action of PE

For the cooking process the following reaction is formulated:

*Cooking:*



This reaction describes the  $\beta$ -degradation of pectin. The galacturonic acids within the pectin polymer are partly methylated and partly unmethylated.  $\beta$ -Degradation causes the polymer to break between adjacent methylated galacturonic acids within the polymer chain. The consequence of this reaction is that during the course of the reaction (=cooking) the DP decreases and results in pectic fragments which are water soluble (WSP) and do therefore

not contribute to the texture of the product. This equation also describes that during this  $\beta$ -degradation the DE is not affected. Eqn. 20 can be converted into a differential equation (eqn. 21a) and solved at constant temperature to give eqn. 21b

$$\frac{\partial DP}{\partial t_c} = -k_\beta \cdot DP \cdot DE \quad (21a)$$

$$DP(t_c) = DP_0 \cdot e^{-k_\beta \cdot DE(t_p)} \quad (21b)$$

Eqn. 21 describes the decrease of DP due to  $\beta$ -degradation as function of cooking time, given the DE resulting as a consequence of the preheating time such as described in eqn. 19.

Combining eqn.19 and eqn. 21 integrates the effect of both the preheating and cooking time and results in:

$$DP(t_c) = DP_0 \cdot e^{-k_\beta \cdot t_c \cdot DE_0 \cdot e^{\frac{k_s \cdot PE_0 \cdot (e^{-k_d \cdot t_p} - 1)}{k_d}}} \quad (22)$$

This triple exponential function is the mathematical description of the combined preheating process (at a given temperature during a given time  $t_p$ ), and cooking process (at a given temperature during a given time  $t_c$ ) and the consequences of these combined processes on the DP at the end of the cooking process (Tijskens *et al.*, 1997c).

Described earlier, the firmness of peaches decreases due to the action of PG. All changes in firmness were ascribed to changes in the DP of pectin. These changes in DP were accordingly converted into eqn. 8 describing the changes in observed firmness on basis of the PG catalysed depolymerisation of the pectin matrix. The results of this analysis, given in Table 11-2, strongly suggests the validity of this approach. In other words, the firmness of plant tissue consists of a fixed (cellulose-hemicellulose based) and variable (pectin based) part. Ascribing the changes in DP to the variable part due to the combined preheating and cooking process results in:

$$\text{Firm} = \text{Firm}_{\text{fix}} + \text{Firm}_{\text{var}} \cdot \frac{DP(t_c)}{DP_0} \quad (23a)$$

In combining eqn. 22 and eqn.23 a mathematical description (eqn. 24) is formulated describing how the variable part of the firmness is affected by both the preheating and the cooking process and consequently the firmness of the product.

$$\text{Firm} = \text{Firm}_{\text{fix}} + \text{Firm}_{\text{var}} \cdot e^{-k_\beta \cdot t_c \cdot DE_0 \cdot e^{\frac{k_s \cdot PE_0 \cdot (e^{-k_d \cdot t_p} - 1)}{k_d}}} \quad (23b)$$

The result of this analysis is compiled in Table 11-5. In Figure 11-8 the actually measured firmness of carrots after cooking preceded by a preheating treatment is given in a three-dimensional representation. Each point in this figure gives the firmness of a sample after cooking, at a given preheating time and temperature. The lines in this figure are the simulated behaviour based on eqn. 23 and the parameter values from Table 11-5. This figure clearly shows that virtually no firming effect is observed by preheating at temperatures either below 40 °C, or above 75 °C. The absence of an observed firming effect below 40 °C is caused by the absence of a noticeable total exerted effect due to PE functioning; the enzyme isn't sufficiently active below this temperature. Above 75 °C the temperature dependent inactivation is fast; the PE is already inactivated before the consequence of its enzymatic action becomes apparent.

At the other hand if the preheating time is short the highest effect is observed at relatively high temperatures; the temperature dependent enzyme activation process prevails the temperature dependent inactivation process. For example, after 30 min at 70 °C, at this preheating temperature the highest firmness is observed. After this period of time at this temperature only 0.1% of the PE is still active. Increasing the preheating time results both in an increase in firmness and a shift in the maximal measured firmness to lower preheating temperatures. Obviously at about 55 °C a balance is reached between the total exerted activity and enzyme denaturation; for example, at this temperature, after two hours about 13% of the enzyme is still active.

The information shown in Figure 11-8 was used as input information for eqn. 23 to estimate the values of the rate constants and activation energies. The results of this

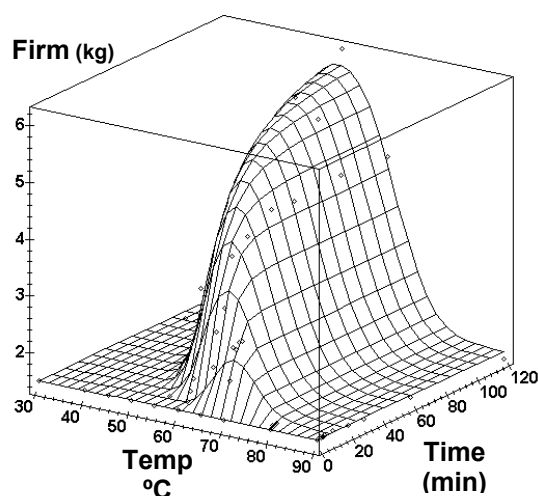


Figure 11-8 Three dimensional representation of the measures (symbols) and simulated (solid lines) firmness of carrots as function of preheating time and temperature followed by a cooking process.

Table 11-5 Results of the statistical analysis for the PE activity in carrots, based on either PE activity measurements (*PE model*) or firmness modulation during preheating and cooking (*BRAM model*)

<i>Parameter</i>	<i>PE model<sup>a</sup></i>		<i>Preheating-Cooking Model (BRAM)</i>	
<b>Estimated parameters</b>				
	<i>Estimate</i>	<i>s.e.</i>	<i>Estimate</i>	<i>s.e.</i>
<i>Kinetic parameters</i>				
$k_{d,sol,ref}$	$4.25 \cdot 10^{-2}$	$4.65 \cdot 10^{-3}$	$4.87 \cdot 10^{-2}$	$2.68 \cdot 10^{-3}$
$Ea_{d,sol}/R$	$1.93 \cdot 10^4$	$9.56 \cdot 10^2$	$1.95 \cdot 10^4$	$4.64 \cdot 10^2$
$k_s$	n.a.	n.a.	$2.42 \cdot 10^{-1}$	$5.71 \cdot 10^{-3}$
$Ea_s/R$	n.a.	n.a.	$1.37 \cdot 10^4$	$2.18 \cdot 10^2$
<i>Batch parameters</i>				
$PE_{sol}$ ; Activity range	9.01 - 10.0	0.24 - 0.62	n.a.	n.a.
$Firm_{var}$ ( $\equiv DP_0$ )	n.a.	n.a.	4.73	$1.59 \cdot 10^{-1}$
<b>Fixed parameters</b>				
<i>Kinetic parameters</i>				
$k_{\beta}$	n.a.	n.a.	$2.3 \cdot 10^{-2}$	n.a.
<i>Batch parameters</i>				
$PE_{sol}$	n.a.	n.a.	1	n.a.
$DE_0$	n.a.	n.a.	$6 \cdot 10^1$	n.a.
$Firm_{fix}$	n.a.	n.a.	1.5	n.a.
<i>Administrative information</i>				
$R^2_{adj}$	89.5		95.4	
$T_{ref}$	60		60	
$t_c$	n.a.		30	
$N_{obs}$	159		294	
<sup>a</sup> .	data derived from Table 11-3			
n.a.	not applicable			

analysis are compiled in Table 11-5.

Several conclusions can be derived from the information given in Table 11-3 and in Table 11-5. Based on the temperature time dependent studies of PE in carrots (*PE model* in Table 11-3; see earlier) estimates could be made for the kinetic value  $k_{d,sol,ref}$  and its activation energy  $E_{a,d,sol}/R$ . Based on firmness measurements of carrot samples preheated at different times and temperatures a model was formulated (*Preheating-Cooking Model ;BRAM*), including both the enzymatic action of PE and the chemical depolymerisation of pectin due to  $\beta$ -degradation (see eqn. 23), describing how the variable part of the firmness is affected by both the preheating and cooking time. Making use of this last model the parameters  $k_{d,sol,ref}$  and  $E_{a,d,sol}/R$  were also estimated. Comparing the values for  $k_{d,sol,ref}$  and  $E_{a,d,sol}/R$  for the *PE model* and for *BRAM* it can be concluded that these values are identical. This conclusion is supported by the values of the explained parts  $R^2_{adj}$  being 89.1% and 95.4% for the *PE model* and *BRAM* respectively.

In conclusion, based on different starting points, respectively structured enzyme (in)activation studies and structured firmness studies, using the same kinetic formulations to describe underlying processes, identical results are obtained. This altogether strongly supports the validity of the approach presented.

### ***A histological, stochastic approach to relate the mode of tissue rupture with rupture stress***

The sensory perceived texture of agro-product comprises several elements, e.g. firmness, crispiness, meatiness, etc. The value of some of the product properties relating to firmness can be measured making use of e.g. compression or tensile measurements. In some cases the results of the instrumental determined firmness relate well with the sensory perceived firmness, such as is the case for e.g. carrots. In other cases this relation is completely absent. For example the potato varieties Nicola and Irene represent with regard to their sensory perceived texture characteristics two extremes. Nicola represents a waxy and Irene a very crumbly, mealy potato. (Van Marle *et al*, 1997). Analysing the tissue softening during cooking by compression measurements shows that the softening process of both varieties can be described by an exponential decay process, with slightly different rate constants, representing the variable part of the firmness (Van Marle, 1997). The fixed part of the firmness is almost identical for both varieties. Whatsoever, this fixed part is perceived by the consumer and is assessed to be completely different between these varieties. In other words, no relation exists between instrumentally determined firmness and sensory perceived texture. This difference can be explained on basis of differences between these varieties at the cellular level. Apparently, the crumbly, mealy potato variety tissue fractures through the cells, the waxy variety fractures along the cells, through the middle lamellae region of the cell walls. The fracture force measured is however in both cases identical. In other words sensory perceived texture attributes are in this example strongly related to the mode of tissue fracture rather than to the required fracture force.

To address this problem, Verlinden *et al.* (Verlinden, 1996; Verlinden *et al.* 1996; Verlinden *et al.* 1997a; Verlinden *et al.* 1997b) modelled the relation between the macroscopic tissue strength and the strength of both the cell wall and the middle lamellae using a stochastic approach. The macroscopic (tissue) strength of a vegetable, in this case carrots, was determined by measuring the mode of rupture and the rupture stress using a special designed ring shaped device (Verlinden, 1996; Verlinden *et al.* 1997b). The measured, macroscopic values represent average tissue properties resulting from the interactions between individual cells of the tissue. The strength of a tissue is determined by the sum of the normalised cell-cell interactions (normalise either per cell, or surface rupture area) and the cell wall strength. Again, the cell-cell interactions are determined by pectin, confined to the middle lamellae region of the cell walls, representing the variable part of the firmness. The cell wall strength is determined by the cellulose-hemicellulose matrix and reflects the fixed part of the firmness. The middle lamellae strength can be defined as the middle lamellae breaking force,  $F_l$ , and the strength of the cell wall as cell wall breaking force,  $F_w$ . The value of the breaking force of either middle lamellae or cell wall depends on its intrinsic

strength and on the dimensions of the middle lamellae or cell wall, respectively. The geometrical arrangement of the cells and their relative orientations (the tissue archestructure) with respect to the applied forces on the tissue also affects the rupture force of a particular cell. Confining this information in a mathematical description gives:

$$F_l = S_l \cdot A_l \quad (24a)$$

$$F_w = S_w \cdot A_w \quad (24b)$$

In eqn. 24,  $S$  stands for the intrinsic strength defined as the rupture stress of the material and  $A$  is the dimensional and geometrical (=archestructural) factor. Each individual cell has its own dimension and geometrical orientation. Furthermore, the cell wall thickness and the properties of the pectin in the middle lamellae have their own biological variability. In taking these considerations into account the cell wall strength,  $F_w$ , and the middle lamellae strength,  $F_l$ , can be considered as stochastic (probably) independent variables, each with its own mean value, ( $\mu_w$  and  $\mu_l$ ) and standard deviation ( $\sigma_w$  and  $\sigma_l$ ). Whenever the middle lamellae strength,  $F_l$ , is higher than the strength of the cell wall,  $F_w$ , the cell will rupture through the cells and vice versa, when  $F_w$  is higher than  $F_l$  the cells will rupture along the middle lamellae. This is mathematically written as follows:

$$F_d = F_l - F_w \quad (25a)$$

$$\text{If } F_d > 0 \Rightarrow \text{cell wall break} \quad (25b)$$

$$\text{If } F_d < 0 \Rightarrow \text{middle lamellae break} \quad (25c)$$

On the rupture surface of the tissue the percentage of cell wall breaks equals the proportion of cell for which  $F_d > 0$ . This proportion can be calculated using the cumulative distribution function of  $F_d$ . In other words, the mode of surface rupture is related to a stochastic process. The next question to be addressed is the relation between the percentage cell wall breaks on the rupture surface and the rupture stress as measured with the tensile test.

Verlinden et. al. worked this problem out into a statistical but dynamic distribution model. With these models it becomes possible to estimate stresses acting on single cells from the macroscopic tissue strength (organ level) properties and the histological properties (cellular level) based on cell sizes and cell wall break data.

The mathematics are, however, too complex to include the complete deduction and validation of these models in the framework of this chapter.

## Acknowledgements

The results of the research presented here were obtained within the framework of the EU-AIR project under project number AIR1-CT92-0278 entitled: "The (bio)chemistry and archestructure of fruit and vegetable tissue as quality predictors for optimising storage and processing regimes: Basic research leading to applicable models and rules", partly financed by the European Union.

The authors wish to express their gratitude to the participants of this project:

K.W. Waldron, Institute of Food Research, Norwich, UK,  
 A.M.M. Stolle- Smits, K. Recourt and C. Boeriu, ATO-DLO, Wageningen, The Netherlands,  
 P.S. Rodis, Agricultural University of Athens, Athens, Greece,  
 B.E Verlinden and J. DeBaerdemaeker, Catholic University Leuven, Leuven, Belgium,  
 T. de Bary and R. Deltour, University of Liege, Liege, Belgium  
 Zarra, University of Santiago de Compostella, Santiago de Compostella, Spain

C. van Dijk was co-ordinator of this project

Table 11-6 List of Symbols

Variable	Meaning	Dimension
A	Geometrical factor	m <sup>2</sup>
Ea	Activation energy	J.mol <sup>-1</sup>
F	Force	N
k	Reaction rate constant	s <sup>-1</sup>
R	Universal gas constant	J.K <sup>-1</sup> .mol <sup>-1</sup>
PE	Activity of PE	nkatal.gFWT <sup>-1</sup>
PG	Activity of PG	nkatal.gFWT <sup>-1</sup>
POD	Activity of POD	nkatal.gFWT <sup>-1</sup>
S	Rupture stress	Pa
t	Time	s
T	Temperature	°C or K
<b>Indices</b>		
abs	absolute temperature	
bnd	bound form	
c	conversion	
d	denaturation	
fix	fixed part	
i	any	
l	middle lamellae	
ref	at reference temperature	
sol	soluble form	
var	variable part	
w	cell wall	
0	initial	

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**12**

**Modelling the Change in Colour of Broccoli and  
Green Beans during Blanching.**

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Published in *Innovative Food Science & Emerging Technologies*, **2**, 2001, 330-313

## Abstract

The green colour of vegetables changes considerably during heat treatments like blanching. Green beans from two different countries and growing seasons, and the stems and florets of broccoli were heat-treated from 40 up to 96 °C. The colour was monitored with the CIE-Lab system. Expressing the green colour as  $-a^*/b^*$  proved to reduce considerably the observed variance within measuring samples. It can be considered as a kind of internal standardisation.

The colour was modelled by a simplified kinetic mechanism of two consecutive reactions, one that increases colour, one that degrades colour. First, all data sets were analysed separately using non-linear regression. The obtained percentage variance accounted for ( $R^2_{adj}$ ) ranged from 75.7 to 90.8%.

Allowing separate initial conditions but with the kinetic parameters in common, the data of the same vegetable type (green beans and broccoli separately) could be pooled and analysed together ( $R^2_{adj}$  87.4 and 77.2% respectively). The kinetic parameters obtained were so similar that a complete pooled and generic analysis was possible even for green beans and broccoli together. These findings greatly validate the developed model and indicate that the formation and degradation of visible colour in vegetables is governed by processes related to the colouring compounds (like chlorophyll and chlorophyllides), irrespective of the vegetables under study.

## Introduction

Heat treatments of vegetables are mostly intended to tenderise the vegetables for consumption or, as a pre-treatment for freezing or canning, to inactivate enzymes and to remove air. The effects that these heat treatments exert on the colour of green vegetables, have been well studied and described in mathematical models. The majority of these studies concerned only a decrease in colour by heat treatments at constant and steady conditions. However, looking in more detail to the initial period of the heating time, a substantial increase in green colour is observed. Some studies have mentioned this phenomenon (Herrmann 1983, Lau et al. 2000, Mackinney and Weast 1940, Meyer 1960), but none of these references have related this increase in initial colour to time and temperature of the heat treatment.

In general, the colour observed by human beings is the perception of the wavelengths coming from the surface of the object on the retina of the eyes. The human eyes are sensible for the wavelengths between 400 and 700 nm with an optimum sensibility for light with a wavelength of about 550 nm. From the spectral characteristics of the surface of an object, the colour can be expressed in variables that correspond with the colour perception of the average human being. One of these sets of variables are the CIE  $L^*$ ,  $a^*$  and  $b^*$  values that expresses the "brightness", the "green-red" and the "blue-yellow" value, respectively. The colour of green vegetables is mainly determined by the chlorophyll pigments present in plant material to catch the energy from sun light. The CIE-Lab system is frequently used as a versatile and reliable method to assess the colour of fruit and vegetables during storage and processing (Gnanasekharan et al. 1992, Tian et al. 1995, 1996, Barrett et al. 2000, Gunawan & Barringer 2000). Kidmose & Hansen (1999) reported a good relation between instrumental colour, sensory yellowness and chlorophyll content in cooked and stored broccoli florets. These chlorophyll pigments degrade during heat treatments, with the consequence that the green colour changes. Weemaes et al. (1999) modelled the thermal degradation of chlorophyll in broccoli, based on separate but concomitant degradation of chlorophyll a and b. The physical colour on the other hand was modelled by a consecutive reaction mechanism, based on the conversion of chlorophyll to pheophytin and further to pyropheophytin. These changes in chlorophyll pigments have been monitored by the ratio of  $a^*$  to  $b^*$  ( $-a^*/b^*$ ) for canned green peas (Gold and Weckel, 1959), for blanched and frozen broccoli (Gunawan and Barringer 2000) and for canned green beans (Hayakawa, 1977).

Furthermore, the expression  $\arctan\left(\frac{b^*}{a^*}\right)$  is a characteristic for the hue angle of the

measured surface. The ratio of  $-a^*/b^*$  is therefore also related to the hue angle. Hue angle has also been used to describe and model colour changes for tomatoes (Tai et al. 1990).

The change in green colour due to heat treatments consists initially in an increase in colour followed by a decrease. This initial increase in green colour has been reported in literature, but not modelled or expressed quantitatively. Most studies on changes in green colour due to time and temperature treatments only mention a decrease of green colour. For an important part this is related to the time scale during which the colour development is studied. Attention is focused to prolonged heating at higher temperatures like e.g. during sterilisation, or the vegetables were already blanched before colour measurements were performed (Gupte et al. 1964, Gunawan & Barringer 2000). Hayakawa (1977) used defrosted green beans for the study of the effect of heating between 80 and 149°C. Some studies concerned fresh vegetables at moderate temperatures (80-120°C) using vegetable juice (Weemaes et al. 1999) or puree (Buckle & Edwards 1970).

Only few authors mention an initial increase in green colour upon heating. Herrmann (1993) reported that blanching times of 30 s resulted in higher scores for the green colour in unblanched green beans. Lau et al. (2000) noticed an initial increase in green colour of green asparagus at heat treatments between 70 and 98°C. Woolfe (1979), Mackinney and Weast (1940) and Meyer (1960) attributed the initial increase of the green colour in green vegetables to air removal around the fine hairs on the surface of the plant and to the expulsion of air between the cells. Both processes were assumed to alter the surface reflecting properties.

At prolonged heating the initial increase of green colour values is followed by a decrease in greenness mainly caused by the formation of pheophytin (Woolfe, 1979) by the exchange of  $Mg^{++}$  by  $H^+$  in the centre of the porphyrin ring of chlorophyll. This degradation of chlorophyll is often studied and described by a simple first order reaction mechanism (Weemaes 1999, Schwartz and von Elbe 1983). Recently models have been reported based on the complete mechanism involved in chlorophyll degradation (van Boekel 1999, 2000, Heaton and Marangoni 1996).

In most studies only the physically observed green colour (expressed as e.g.  $a^*$  or  $-a^*/b^*$ ) is measured and analysed. Van Boekel (1999), however, advocates a multiresponse approach, where the concentrations of all colouring compounds occurring in chlorophyll degradation in foods (chlorophyll, chlorophyllides, pheophitines etc.) are included simultaneously in one analysis. This approach considerably improves the statistical fit, the precision and reliability of parameter estimates and the available knowledge and interpretation of the data.

Van Boekel (1999, 2000) and Heaton and Marangoni (1996) directed their effort to concentration of colouring compounds, and included all pathways possible in fresh produce. In this study attention has been devoted to describing and modelling the behaviour of physical colour during blanching, as observed by the human eye, based the complex mechanisms occurring (Van Boekel 1999, 2000, Heaton and Marangoni 1996), but appropriately simplified to comprise only mechanisms active during the blanching process. However, the commonly observed increase in perceived colour at the very early stages of blanching, has to be incorporated to be able to eventually optimise the blanching process with respect to retained colour.

## Materials & Methods

### *Product*

The experiments on green beans were conducted twice (April 1999 and August 1999). In April 1999, the experiments were conducted using green beans (cv. Bronco) imported from Senegal. For the August 1999 experiments, green beans (cv. Masai) were grown in the Flevopolder in the Netherlands. During the experimental period, the beans were stored at 6 °C (the lowest so-called safe temperature).

Broccoli (cv. Marathon) was grown in 1999 in Raamsdonkveer in the Netherlands and harvested in June. During the experimental period, the broccoli was stored at 1 °C, covered with foil.

## Methods

### Product preparation

The pulses of the green beans were split to promote heat diffusion and reduce the effect of inhomogeneous heating.

Broccoli florets of about 5 cm length were cut from the main stem. Subsequently, the florets were split length-wise into parts with a thickness of not more than 5 mm. The broccoli stems were longitudinally cut in halve to promote heating homogeneity, and to facilitate the colour measurement.

### Heating and cooling

For each combination of time and temperature, as mentioned in Table 12-1, one sample (350 g of beans or 750 g of broccoli) was soaked in 20 l water at the temperature under study. To avoid an unacceptable temperature drop at the start of the experiments, fresh water was used for each sample. The treatments at a particular temperature were performed after each other, but the order of the residence time was randomly distributed. The heating vessel was equipped with an electric heating unit of 650 W with temperature control ( $\pm 1$  °C) and stirred by a propeller, diameter 110 mm, driven with a 120 W motor in 890 rotations per minute. During the heat treatments, the temperature of the water was recorded with thermocouples. The time schedule applied for heating the samples was adapted to the temperature applied. In Table 12-1 the residence times for the broccoli experiments are shown. Similar residence times were used for green beans. The residence time of the samples in the vessel was checked manually by means of a stopwatch.

Table 12-1 Time-temperature combinations applied to heat the samples of broccoli

		Temperature ( °C)						
		40	50	60	70	80	90	96
Time in seconds	0	0	0	0	0	0	0	0
	30	15	5	5	5	5	5	5
	60	30	10	10	10	10	10	10
	150	60	30	30	20	20	20	20
	300	180	100	60	40	30	30	30
	600	420	300	120	60	40	40	40
	1350	900	600	240	150	60	50	50
	2700	1800	1200	420	300	120	60	60
	5400	3600	2400	900	600	240	120	120
	10800	7200	3600	1800	900	450	240	240

At the end of the heating time, the sample was immediately cooled during 5 minutes in water of 0 to 6 °C. After cooling, the samples were dried first by swinging out and afterwards by spreading out on paper towels.

The samples of green beans were laid down side by side with the split surface down on a black plate of PVC to form as good as possible a flat surface. On this carpet of beans, the colour was measured at 20 spots with a Minolta CR200.

For the colour measurement of broccoli, the florets and stems were clamped manually to the orifice of the Minolta CR200. Both for the florets as for the stems of broccoli, 20 spots were measured for each time-temperature combination. The colour of the broccoli stems was measured on the cut surfaces of the stems.

### Statistics and modelling

Measurements at each time-temperature combination were repeated 20 times on different parts of the samples. The measured CIE  $a^*$  and  $b^*$  values were transformed into their ratio



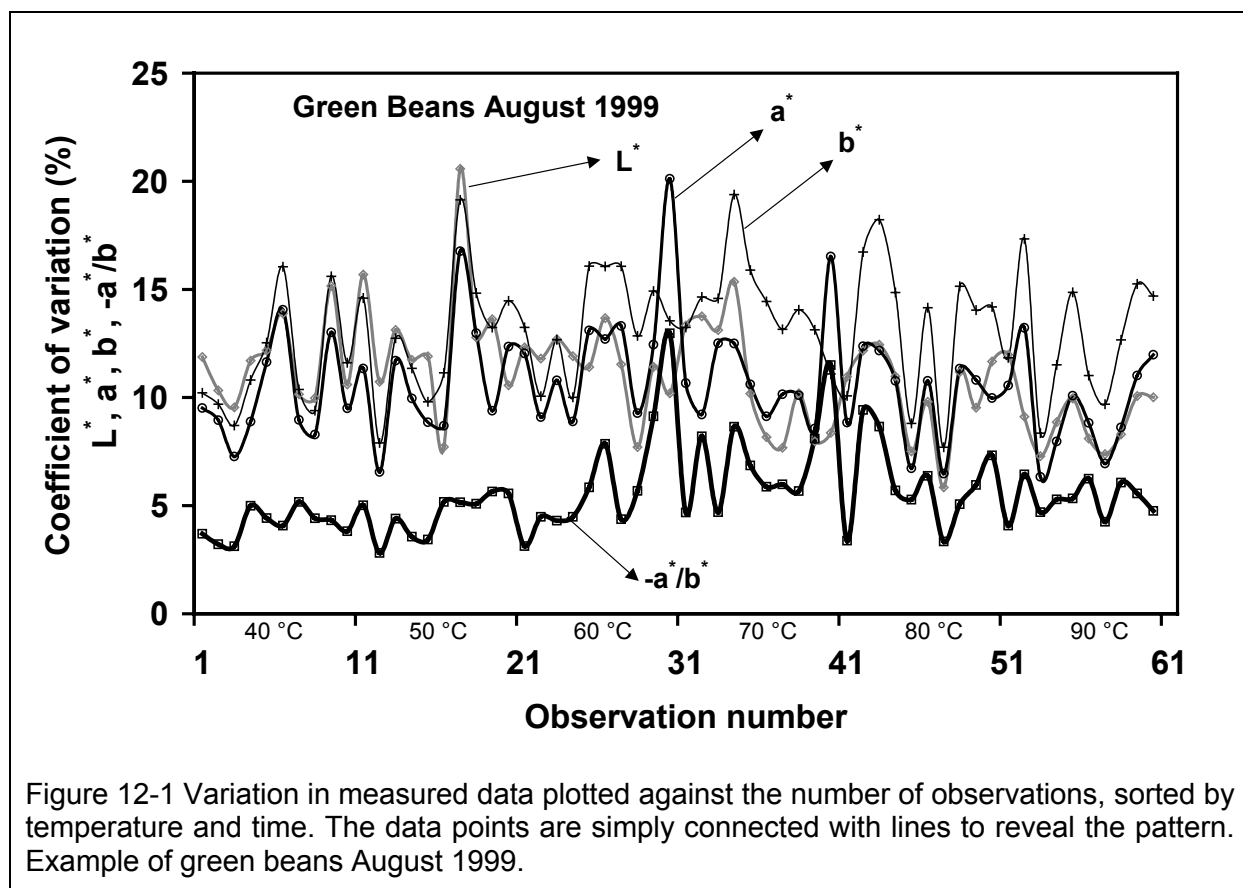
$-a^*/b^*$ . Since the samples can not be used again for another measurement in time (samples were discarded after measurement), the measuring technique can be regarded as destructive. As a consequence the variation between the samples are rather large. The mean colour was therefore used for model development and further statistical analysis. This technique has of course the consequence that the obtained reliability is valid for the mean data only. For the original raw data the reliability will be considerably less. Statistical analyses were conducted using the non-linear regression package of Genstat (Rothamsted, UK). In all analyses a weight factor was applied to the data, equal to  $1/\text{stdev}^2$ , with stdev the observed standard deviation over the replicates within a sample.

The model was developed based on plausible occurring processes rather than directly modelling the phenomena observed in the data. The model was developed using MapleV R4 (Waterloo Maple Inc., Waterloo, Canada), a computer program capable of handling symbolic functions and equations.

## Results and Discussion

### Raw Data

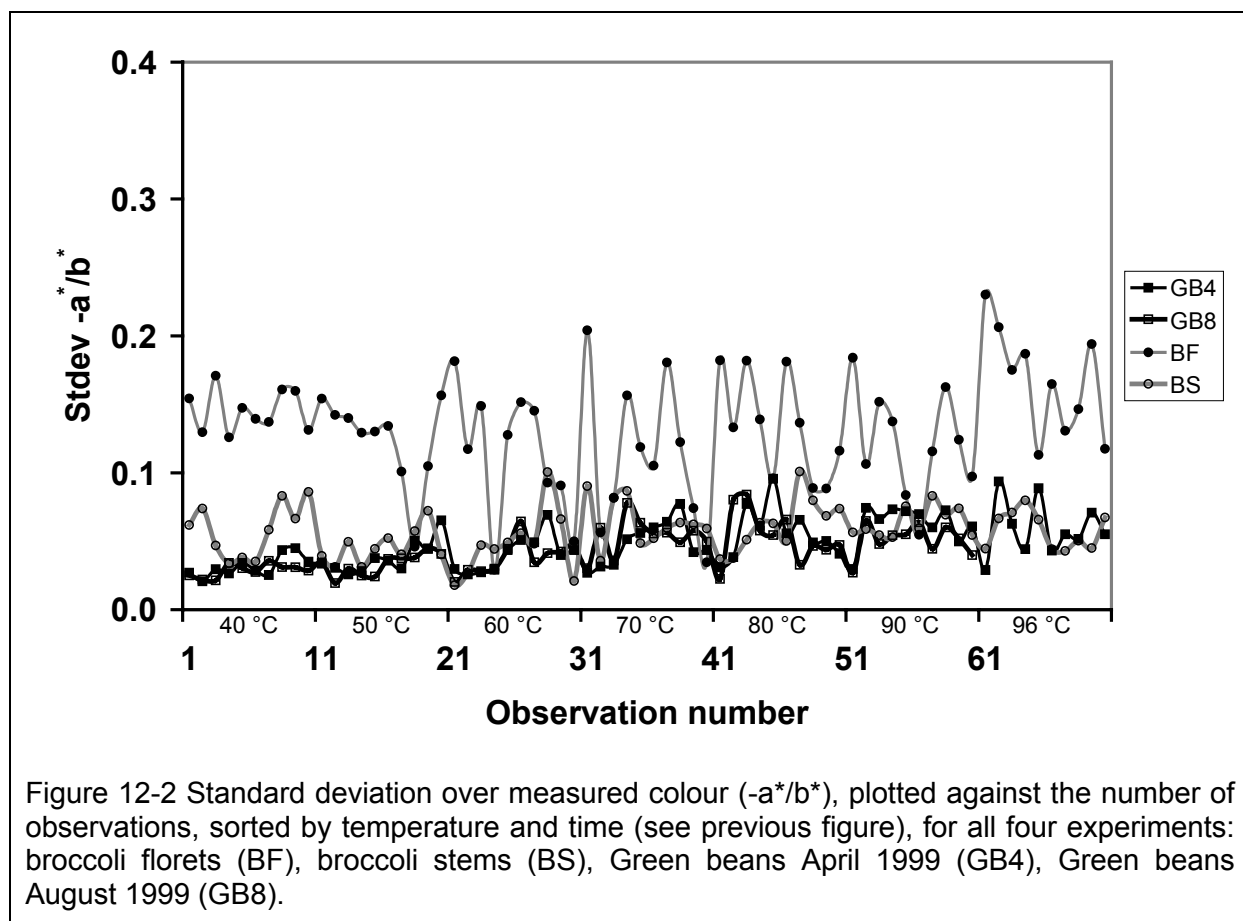
The colour of the samples was measured in the CIE-Lab system. The behaviour of the  $L^*$ ,  $a^*$  and  $b^*$  values for the different samples was, however, very much masked by the large variation within and between the samples, partly due to the use of different fresh samples for each temperature-time combination. In Figure 12-1, an example for the variation, expressed as coefficient of variation ( $100 \times \text{stdev}/\text{mean}$ ), is shown. The behaviour of the coefficient of variation in time at the different temperatures is comparable for all three variables  $L^*$ ,  $a^*$  and  $b^*$ , and for both products studied. To apply a kind of internal standardisation for the variation, the ratio of  $-a^*/b^*$  was used to describe the colour behaviour. This approach has already been described and used (Gold and Weckel 1959, Hayakawa 1977, Gunawan & Barringer 2000). The resulting variation of  $-a^*/b^*$  is also shown in Figure 12-2. Since  $-a^*/b^*$  is a relative value, related to the colour hue, it is more or less independent of the variation in absolute



reflection and the coefficient of variation of  $-a^*/b^*$  is considerably smaller than that of the constituting variables. Due to the small numerical value of  $-a^*/b^*$  compared to the numerical value of  $a^*$  or  $b^*$ , the absolute standard deviation (stdev) of  $-a^*/b^*$  is even considerably smaller. This transformation of data thus ensures that the major part of variance in the successive samples, due to the measuring circumstances, is eliminated. The order of magnitude of the standard deviation for the four types of samples can be taken from Figure 12-2. It also shows an obviously higher standard deviation for broccoli florets, as compared to the other three sets of data.

The behaviour of the colour of green beans (experiment August 1999) is shown in Figure 12-3. The general pattern of the behaviour of colour in the other experiments of green beans and of broccoli was similar. From Figure 12-3, it is clear that colour does increase in level at the very early periods of blanching. The higher the temperature of blanching, the higher the increase in colour, but at the same time, the sooner the colour starts to decay. The result of the combined action of formation and decay is a shift in location of maximal colour to increasingly smaller times at increasingly higher temperatures.

What the reason is for this increase in colour due to short blanching treatments is not exactly known. It could be an effect of physical changes in the vegetable matrix, due to e.g. decreasing opacity by replacement of intercellular air by blanching water and cell juice released by cell membrane deterioration (Woolfe 1979, Mackinney and Weast 1940, Meyer 1960). However, it could also be possible that in the fresh produce some non-coloured or less-coloured precursor of green colour is present that is converted in to more visible green colour intensity upon blanching treatment. This could be taken from the reported mechanisms involved in chlorophyll degradation (van Boekel 1999, 2000, Heaton and Marangoni 1996). Another possible explanation could be that the heat treatment gradually destroys the cell membranes, resulting in a possible contact between the enzymes and the chlorophyll precursor compounds present in different organelles. In either case, the overall effect is that the green colour increases during the first short blanching periods. The



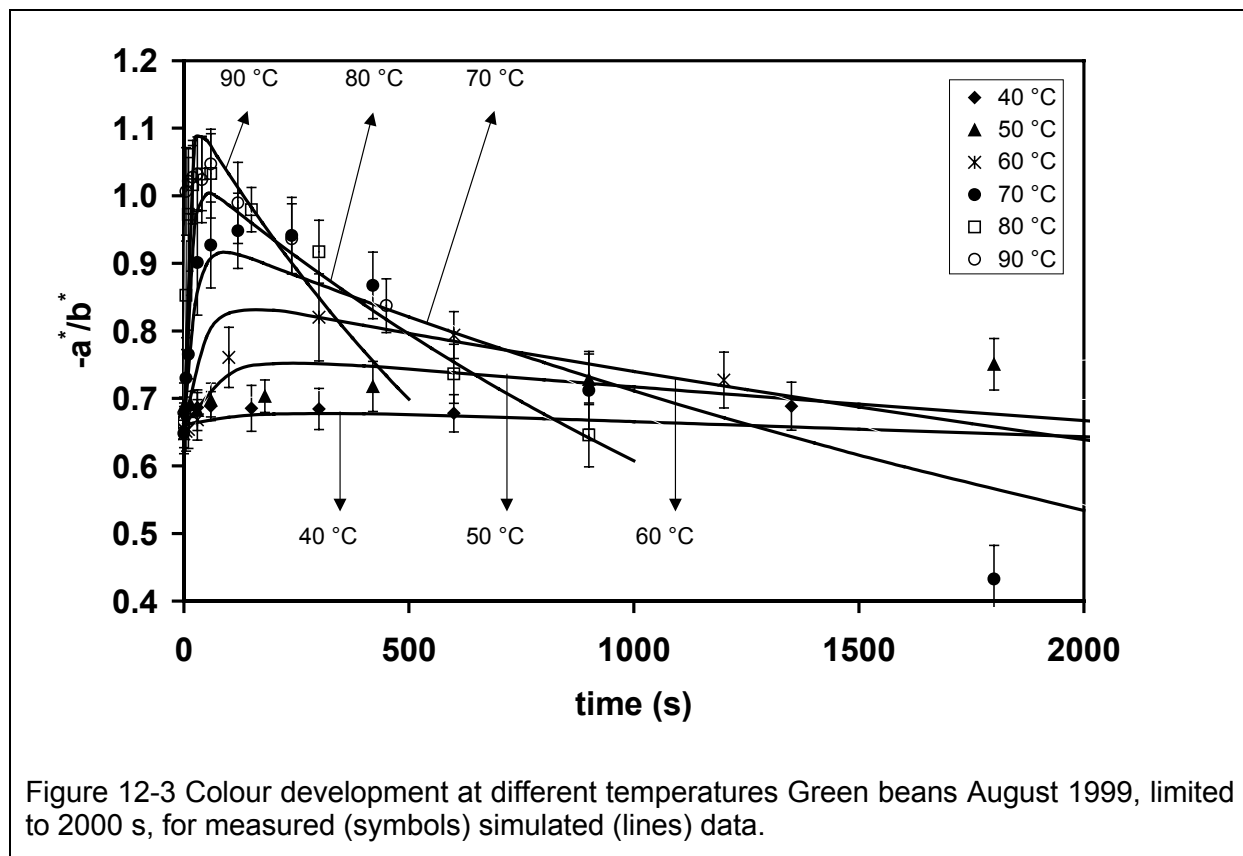


Figure 12-3 Colour development at different temperatures Green beans August 1999, limited to 2000 s, for measured (symbols) simulated (lines) data.

decrease in colour later in the blanching treatment can be attributed to a chemical decay of the green components (chlorophyll) (Weemaes et al. 1999), together with a loss of the liberated colouring compounds by migration into the blanching water.

### Model development

Whether the increase in colour intensity at the early stage of blanching can indeed be attributed to a chemical conversion, or to a physical process connected e.g. with the exhaustion of air, a direct resemblance with consecutive reactions is immediately evident. This type of mechanism can be represented as:



In this mechanism,  $G_p$  can be considered as the colouring compound in a different physical (opaque) precursor configuration from which the colouring compound  $G$  is formed.  $k_c$  and  $k_d$  are the reaction rate constants for the conversion and degradation reaction. Based on the fundamental rules of chemical kinetics, this reaction mechanism can be converted into a set of differential equations:

$$\begin{aligned}
 \frac{dG_p}{dt} &= -k_c \cdot G_p \\
 \frac{dG}{dt} &= k_c \cdot G_p - k_d \cdot G
 \end{aligned}
 \tag{2}$$

This set of differential equations can be solved analytically for constant external conditions since the temperature was kept constant during the blanching experiments. This results in the function describing the concentration of precursor and green colour (eq (3)). Since the precursor  $G_p$  can not be measured, only the equation for the green colour  $G$  is of interest for this study.

$$G_p = G_{p,0} \cdot e^{-k_c \cdot t}$$

$$G = G_0 \cdot e^{-k_d \cdot t} + G_{p,0} \cdot \left( \frac{k_c}{k_c - k_d} \right) \cdot (e^{-k_d \cdot t} - e^{-k_c \cdot t}) \quad \text{eq (3)}$$

where the index 0 refers to the initial state at time zero. In all these equations the reaction rate constants ( $k_c$  and  $k_d$ ) depend like for any normal chemical and enzymatic reaction, on temperature according to Arrhenius' law:

$$k_i = k_{i,\text{ref}} \cdot e^{\left( \frac{E_{a_i}}{R} \cdot \left( \frac{1}{T_{\text{ref}}} - \frac{1}{T} \right) \right)} \quad \text{eq (4)}$$

In this equation  $T$  stands for the temperature (K),  $k$  for any reaction rate constant, index  $\text{ref}$  refers to an arbitrarily chosen reference temperature (60 °C),  $R$  is the universal gas constant (8.314 J/mol) and  $E_a$  is the energy of activation. The model developed (eq. 3) still can not be used to analyse the data obtained. Eq. 3 represents the behaviour of the chemical compound responsible for the green colour (e.g. chlorophyll and or chlorophyllide). As a consequence, its value can never drop below zero. The data measured in the CIE-Lab system are the physical representation of what the human eye can observe (colour). In the CIE-Lab system the  $-a^*/b^*$  value can very well be negative. By translation, eq. 3 can be converted into an applicable equation, representing the physically observed colour (eq. 5).

$$ab = ab_0 \cdot e^{-k_d \cdot t} + ab_{p,0} \cdot \left( \frac{k_c}{k_c - k_d} \right) \cdot (e^{-k_d \cdot t} - e^{-k_c \cdot t}) + ab_{\text{fix}} \quad \text{eq (5)}$$

where  $ab$  represent the value  $-a^*/b^*$ . The time at which a maximal colour development will occur at any treatment temperature can easily be deduced from this model. Calculating the derivative of eq 5 with respect to time, and solving this derivative for zero change (the time of maximal colour) one obtains:

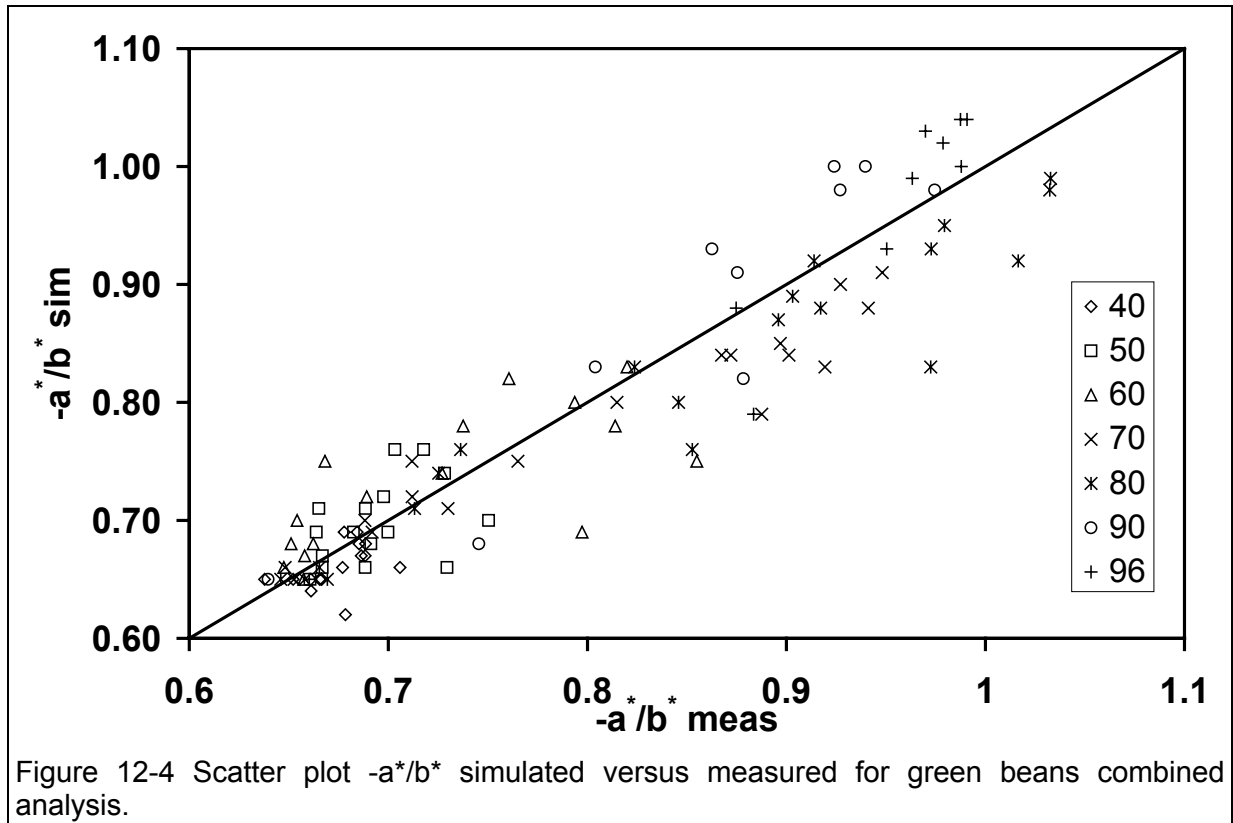
$$t_{\text{max}} = - \frac{\ln \left( \left( \frac{k_c - k_d}{k_c} \right) \cdot \frac{k_d}{k_c} \cdot \frac{ab_0}{ab_{p,0}} + \frac{k_d}{k_c} \right)}{k_c - k_d} \quad \text{eq (6)}$$

Since both  $k_c$  and  $k_d$  depend on temperature according to eq 4, eq. 6 expresses the dependence of the time at which colour develops to maximal values, as a function of treatment temperature.

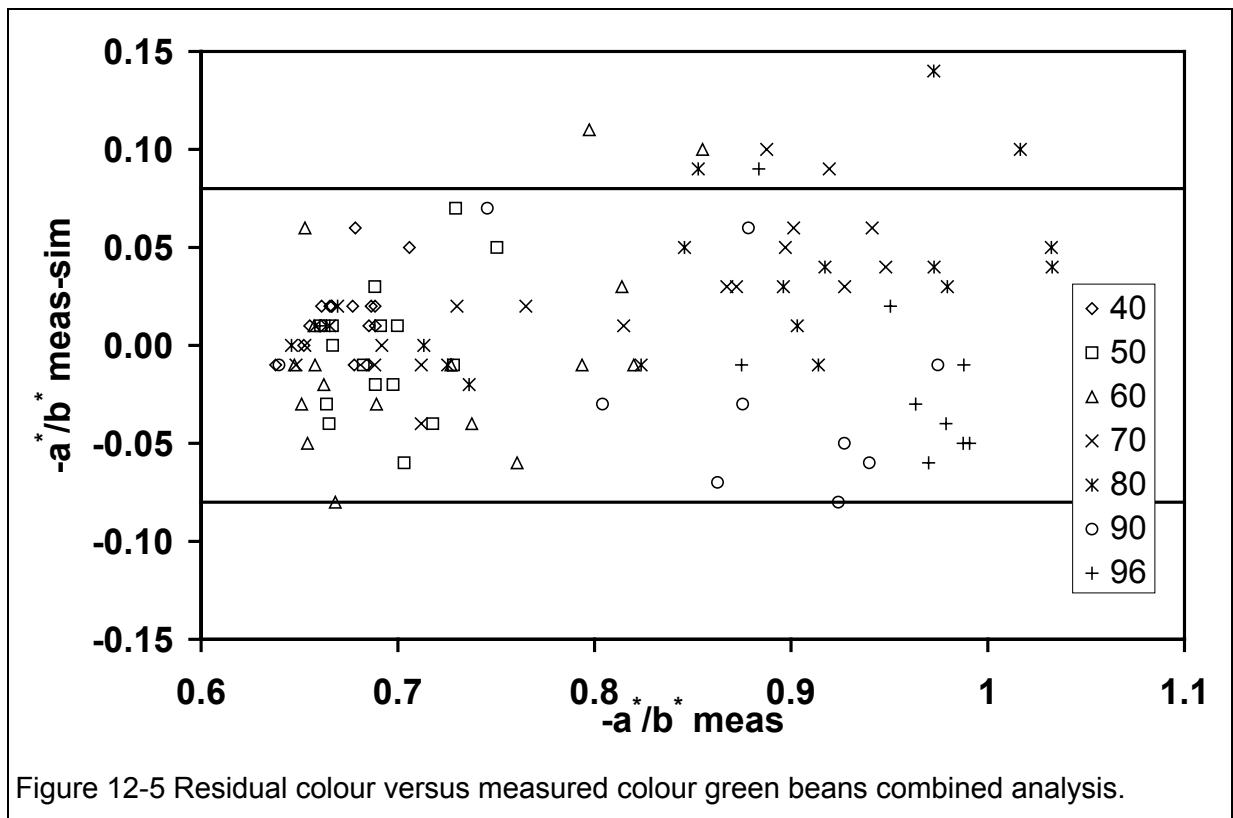
### **Separate statistical analysis**

The colour data, expressed as  $-a^*/b^*$ , of the four series of experiments (two on green beans, one on broccoli florets, one on broccoli stems), were analysed separately without applying further data transformation. The statistical analyses were conducted using the non-linear regression analysis procedure based on the combined equations 5 and 4. A weight factor of  $1/\text{stdev}^2$  was applied in each of the statistical analyses (Tijsskens et al. 2001, van Impe et al. 2001). To avoid too much influence of long tailing especially at low temperatures (see Figure 12-3), analysis was restricted to time values less than 3000 min, excluding effectively only 5 measurements for each subset data.

Preliminary analyses revealed that in these experiments, the value of  $ab_{\text{fix}}$  consistently approximated a zero value. The value of this parameter was therefore fixed to zero, and further excluded from non-linear regression analysis. Since non-linear regression analysis applies an iterative procedure, good and reliable initial estimates are necessary to start to procedure towards a reliable final estimation. The model formulation is too complex and the variation in the data is too large for a direct estimation of all parameters. A stepwise procedure is therefore followed, first estimating the kinetic parameters ( $k_{c,\text{ref}}$ ,  $E_c$ ,  $k_{d,\text{ref}}$  and  $E_d$ ) using plausible values for the batch parameters ( $ab_0$  and  $ab_{p,0}$ ). Subsequently the parameters

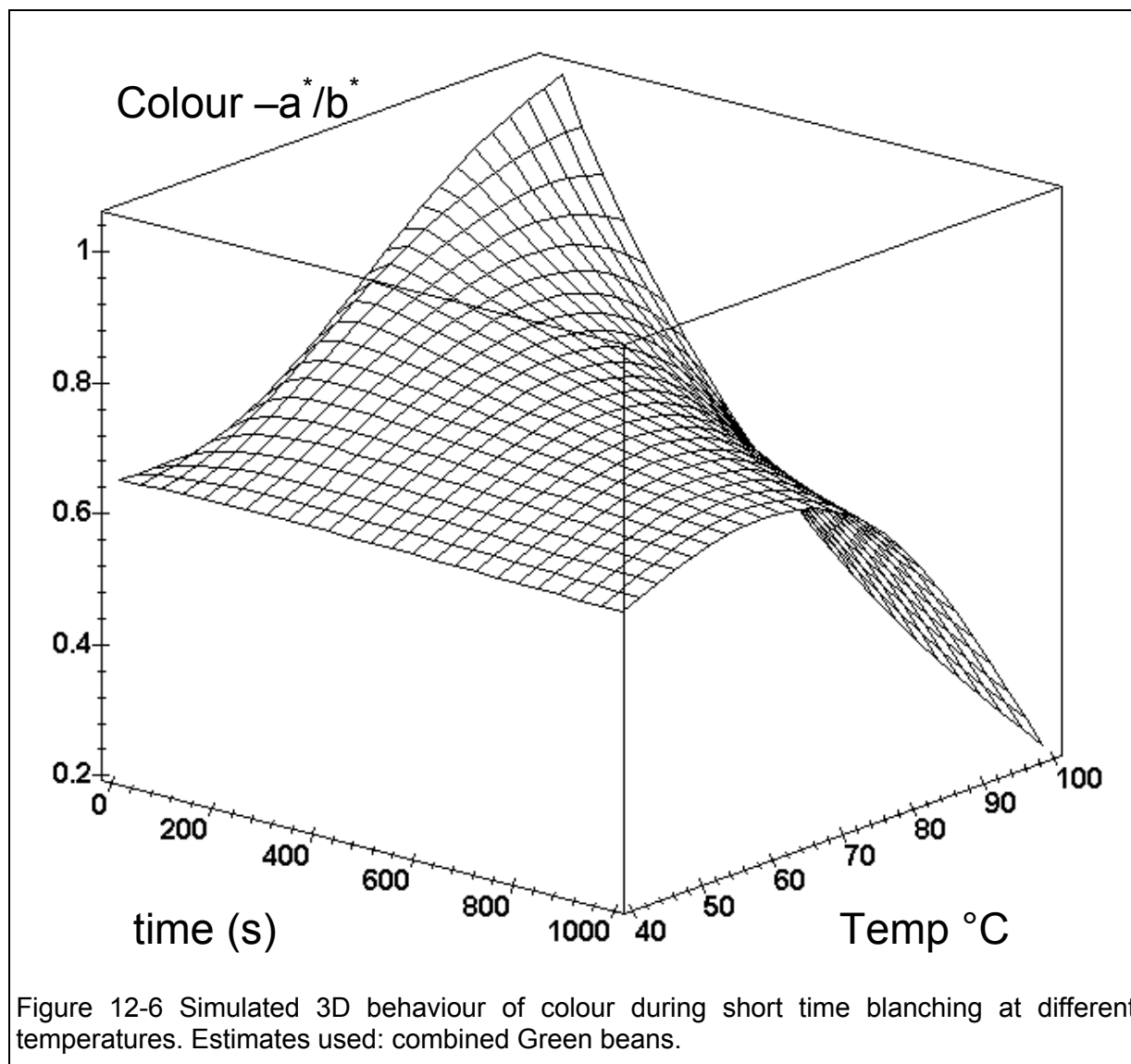


$k_{c,ref}$ ,  $k_{d,ref}$ ,  $ab_{p,0}$  and  $ab_0$  were estimated, keeping the values of both energies of activation ( $E_c$  and  $E_d$ ) fixed at the just obtained value. This cycle was repeated three times. For the final analysis, all parameters were estimated simultaneously with the just obtained values as initial values. The results of the analyses are shown in Table 12-2.



**Pooled analysis**

The parameters estimated for the two experiments on green beans are so similar in value that the combined data could be analysed together (results not shown). The parameters estimated for broccoli florets and stems are too dissimilar to analyse them together without further precautions. Here, the effect of the two types of plant parts is too large to neglect. However, when the proposed mechanism truly reflects the processes occurring in vegetables, these processes and the model description should be the same in any batch of that same vegetable. Consequently, the kinetic parameters ( $k_{c,ref}$ ,  $E_c$ ,  $k_{d,ref}$  and  $E_d$ ) should be common for every batch, while the batch parameters ( $ab_{p,0}$  and  $ab_0$ ) should be estimated separately for each batch of vegetables. This additional consequence of fundamental models allows to pool data from different sets. Pooling data from different experiments has a number of advantages. In the first place, the number of observations increases considerably, while the number of parameters to be estimated does not increase as much. In the second place, part of the variation inherently contained in the data, is lost during the analysing sequence used in classical sequential analysis (e.g. each temperature series separately). By pooling data and using integral non-linear regression analysis, the variation can be used in full without any loss and attributed to and distributed over its specific origins like time, temperature or initial conditions. The final advantage is that expert knowledge, the rules of the actual mechanism and the temperature dependence of rate constants, can be forced upon the statistical estimation system.



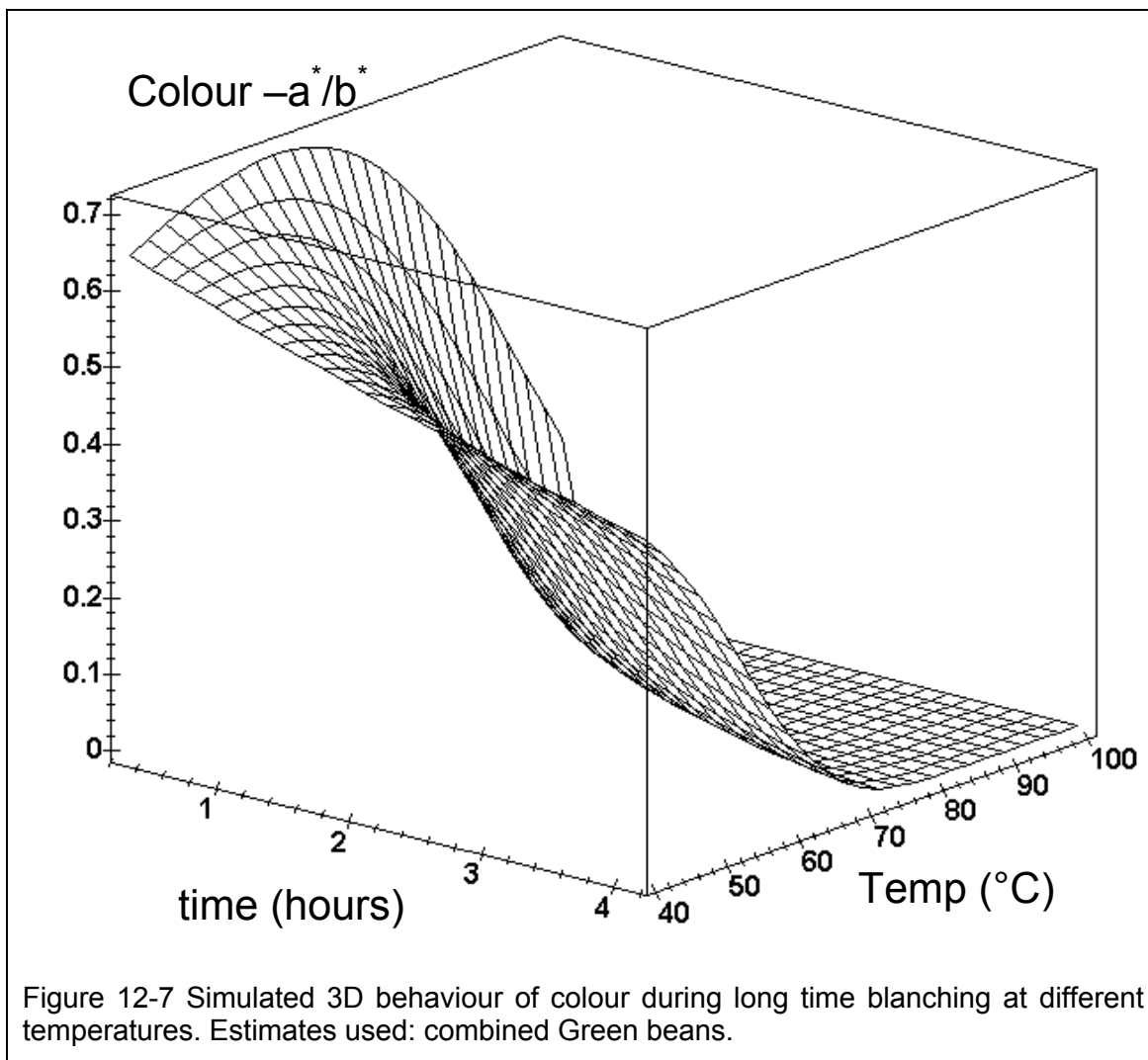
The consequence of all these advantages is, that the reliability and the generic applicability of the model, its estimated parameters and the simulations based on them are greatly enhanced and extended. The data of green beans could already be analysed completely pooled, without the need of separate initial conditions of  $ab_0$  and  $ab_{p,0}$  with a comparable level of the explained part ( $R^2_{adj}$ , not shown). In the case of broccoli, however, it was not possible to analyse directly all data pooled. By allowing for separate initial conditions, a major increase in percentage explained part could be obtained for the pooled data of broccoli stems and florets, so much so that the reliability was

Table 12-2 Results of statistical non-linear regression analysis

	Green beans April 1999		Green beans August 1999	
	Estimate	s.e.	Estimate	s.e.
$ab_0$	0.65197	0.00684	0.6609	0.0107
$ab_{p,0}$	122.7	25.9	136.5	37.7
$ab_{fix}$	0	fixed	0	fixed
$k_{c,ref}$	0.000109	1.33E-05	0.000147	2.23E-05
$E_c/R$	8539	594	7625	734
$k_{d,ref}$	0.0166	0.00338	0.02356	0.00587
$E_d/R$	7615	575	6491	709
$N_{obs}$	65		55	
$R^2_{adj}$	90.8		86.2	
	Broccoli florets June 1999		Broccoli stems June 1999	
	Estimate	s.e.	Estimate	s.e.
$ab_0$	0.7021	na	0.55859	0.00611
$ab_{p,0}$	26.78	na	123.6	69.3
$ab_{fix}$	0	fixed	0	fixed
$k_{c,ref}$	0.000272	na	9.99E-05	1.99E-05
$E_c/R$	6445	na	3036	1320
$k_{d,ref}$	0.00879	na	0.0204	0.0104
$E_d/R$	5873	na	2459	1271
$N_{obs}$	65		65	
$R^2_{adj}$	84.1		75.7	
$T_{ref}$	60			
na	not available			

Table 12-3 Results of non-linear regression analysis of pooled data.

	Green beans Combined		Broccoli Combined	
	estimate	s.e.	Estimate	s.e.
$ab_{0,GB4}$	0.6505	0.00878		
$ab_{0,GB8}$	0.66199	0.00859		
$ab_{0,BF}$			0.7483	na
$ab_{0,BS}$			0.5615	na
$ab_{p,0,GB4}$	130.5	23.7		
$ab_{p,0,GB8}$	139.1	25.1		
$ab_{p,0,BF}$			3.399	na
$ab_{p,0,BS}$			3.233	na
$k_{c,ref}$	0.000126	1.29E-05	0.000300	na
$E_c/R$	8037	496	6452	na
$k_{d,ref}$	0.020570	0.00344	0.001362	na
$E_d/R$	7022	480	5293	na
$N_{obs}$	120		130	
$R^2_{adj}$	87.4		77.2	
$T_{ref}$	60			
na	not available			
BF	Broccoli florets			
BS	Broccoli stems			
GB4	Green beans April 1999			
GB8	Green beans August 1999			



again in the region of the analyses of broccoli stems and florets separately. The results of the pooled non-linear regression analysis are shown in Table 12-3. The scatter plot (simulated versus measured colour) for the combined green beans experiments is shown in Figure 12-4. In Figure 12-5 the corresponding residuals between measured and estimated colour is shown as a function of measured colour  $-a^*/b^*$  value.

In Figure 12-6, the behaviour of the colour of green beans is shown, as simulated, based on the parameters of the combined analysis (Table 12-3) for the short blanching times with identical values at time zero. At longer blanching times, the behaviour tends towards a normal exponential decay, starting, however apparently from different origins (see Figure 12-7). This apparent different initial values result from the very rapid and steep increase in perceived colour by the very rapid conversion of less-green precursor into a bright green compound.

Based on eq. 6, the time of maximal colour development was simulated as shown in Figure 12-8 as a function of treatment temperature for green beans and for broccoli (Table 12-3 combined analysis). For green beans, the derived function (eq.6) proved to be undetermined below 38.5 °C for this set of parameters, since no maximal colour could be detected at low temperatures. From 39 °C on it rises very steeply up to reach a maximum at about 44 °C. Above that temperature a slow decrease can be observed. For broccoli, fundamentally the same behaviour is obtained, except that now the derived function is undetermined below approximately 62 °C and the maximum is reached at about 75 °C.

### **Generic colour decay**

On closer examination of the kinetic parameters ( $k_{c,ref}$ ,  $E_c/R$ ,  $k_{d,ref}$  and  $E_d/R$ ) of the pooled analysis of green beans and broccoli (Table 12-3), one can clearly see the resemblance of



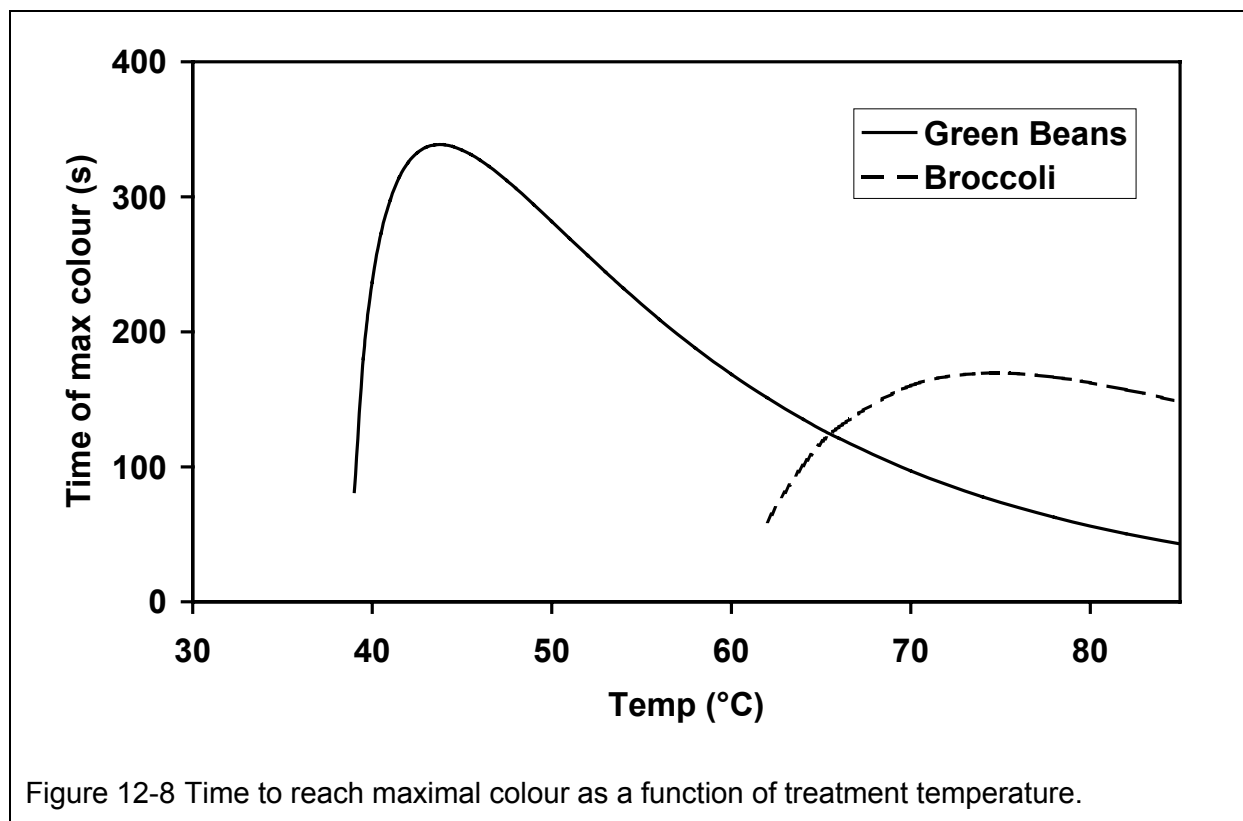


Figure 12-8 Time to reach maximal colour as a function of treatment temperature.

the value of these parameters. If these parameters do have the same value for the two very different vegetable products, it would signify that the same fundamental process, not only with the same mechanism but also with the same parameter values, occurs in both vegetables alike. In a further non-linear regression analysis, the four sets of data were pooled together, and analysed using the model as describe by eqs. 4 and 5, allowing a separate value for the parameters specific for each separate batch: the initial conditions of  $ab_0$  and  $ab_{p,0}$ . In Table 12-4 the results of the generic analysis are shown.

### Meaning of parameters

Building models based on chemical mechanisms automatically provides a fundamental meaning to the model parameters. These can be subdivided into kinetic parameters and batch parameters. Kinetic parameters should be independent of a specific composition of a batch or sample of produce. They express the properties of the occurring chemical processes, irrespective of the coincidental situation in a particular batch. Batch parameters described the actual composition of the compounds, involved in the chemical process, in a specific batch.

### Kinetic parameters:

- $k_{c,ref}$  the reaction rate constant at reference temperature for the conversion of colour precursor into visible colour compounds.
- $E_c$  the temperature sensitivity of that conversion.

Table 12-4 Result of generic analysis

	All data of green beans and broccoli combined	
	estimate	s.e.
$ab_{0,BF}$	0.7262	0.02990
$ab_{0,BS}$	0.5554	0.00996
$ab_{0,GB4}$	0.6501	0.00822
$ab_{0,GB8}$	0.6617	0.00805
$ab_{p,0,BF}$	104.3	16.6
$ab_{p,0,BS}$	87.3	13.9
$ab_{p,0,GB4}$	119.3	18.6
$ab_{p,0,GB8}$	126.8	19.6
$k_{c,ref}$	0.000134	1.09E-05
$E_c/R$	7452	409
$k_{d,ref}$	0.019830	0.002880
$E_d/R$	6525	392
$N_{obs}$	250	
$R^2_{adj}$	85.9	
BF	Broccoli florets	
BS	Broccoli stems	
GB4	Green beans April 1999	
GB8	Green beans April 1999	

$k_{d,ref}$	the reaction rate constant at reference temperature for the decay of colour compounds.
$E_d$	the temperature sensitivity of that decay.

**Batch parameters**

$ab_{p,0}$ & $ab_{p,0,*}$	the initial perception of the colour precursor, present in that particular batch.
$ab_0$ & $ab_{0,*}$	the initial perception of colour compounds, present in that particular batch.

**Discussion**

The measurement of the colour of green beans is difficult. Green beans are too small to be measured separately. Therefore, in a series of parallel placed beans, several individuals were taken for one single measurement. The surface, however, of these adjacent beans is not flat. The curvature between the individual beans causes excessive light scattering and trapping of false light coming in from the environment. In view of this fact, the obtained reliability (high  $R^2_{adj}$  and low standard error of estimates s.e.) is acceptably high. The data from two separate experiments on green beans, from completely different cultivars, origin and growing conditions, could be analysed together without substantial loss of reliability, regardless of allowing for separate initial conditions. This signifies that the proposed model is very likely to be an acceptable simplification of the processes occurring in reality. It also implies that the initial conditions of the green colour in beans are constant throughout the season and irrespective of the cultivar, and that the developed model, including the estimated parameters, can very well be applied in practice.

Measuring the colour of broccoli is even more difficult. Here the sample area is large enough for separate measurements, but the surface of broccoli florets is intrinsically not flat at all, and depends on the ripening stage of the broccoli (e.g. opening stage of the florets depends on ripening stage). Nevertheless, the obtained percentage variance accounted for ( $R^2_{adj}$ ) reflects a reliability comparable to that obtained for green beans (see Table 12-2). What the reason is for the bad reliability obtained in the analysis of broccoli stems (75.7%) is not clear. The surface cut through the stem (before blanching) was flat enough to ensure technically a good measurement. Also the level of  $a^*$  and  $b^*$  was in the same order of magnitude as for the broccoli florets. The standard deviation over the 20 individual measurements of CIE-Lab colour values was for green beans and broccoli stems of the same order of magnitude (see Figure 12-2). For broccoli florets the standard deviation was much larger. So, about the same information regarding colour value and deviation among individuals should be present in the colour data of broccoli stems.

The initial conditions for the batch parameters (Table 12-2,  $ab_0$  and  $ab_{p,0}$ ) were clearly too different to pool directly. However, allowing for initial condition separate for stems and florets, allowed to analyse both type of data together, with a higher  $R^2_{adj}$  then obtained for the stems separately, but lower as compared to the florets separately (see Table 12-3). This signifies that the mechanism and the value of the kinetic parameters are indeed the same for florets and stems. Although, as already mentioned, the overall reliability of the data analyses is not too high, the fact that data can be pooled and analysed together with the same model and common kinetic parameters constitutes a major validation of the proposed model.

For green beans the values for  $ab_{0,*}$  and  $ab_{p,0,*}$  are quite consistent over the successive analyses, building up complicity. This is also the case for the  $ab_{0,*}$  values found for broccoli. For  $ab_{p,0,*}$  values of broccoli the picture is quite the opposite. Apparently the uncertainty in the data with respect to the model parameters is too large to provide a decisive result. This also indicates that more than one solution for the parameter estimates are possible for the same set of data analysed with the same model structure.

Further corroboration for the generic nature of the colour decaying process during blanching treatments, can be found in the complete pooled analysis of all four data sets together, allowing for initial conditions separate for the individual batches (see Table 12-4). These findings would signify that the process of colour decay during heat pre-treatments is truly generic in nature and fundamentally the same for two completely different types of vegetables. Another possible consequence of this type of pooled analysis is that the decay of

chlorophyll and other related colouring compounds during heat pre-treatments is probably not of an enzymatic nature. It is hard to believe that enzyme levels would be the same for all four batches, and different enzyme levels present in the product would induce different apparent colour decaying rates. This deduction on its turn would strongly support the processes to be of a physical nature, rather than a chemical one.

## Conclusions

Expressing CIE-Lab values in terms of  $-a^*/b^*$ , a kind of internal standardisation is applied, that greatly reduces the variation inherently present in such inhomogeneous products as green beans and broccoli.

The development of the green colour, expressed as  $-a^*/b^*$  can be described by a model of consecutive reactions including a (physical) process of formation and a (chemical) process of degradation. Analysis of experimental data based on this model formulation results in reliable estimates ( $R^2_{adj}=87.4$ ) for pooled green beans and a moderate reliability for pooled broccoli ( $R^2_{adj}=77.2$ ).

Despite the higher variability in data of broccoli florets, compared to broccoli stems, the obtained reliability for broccoli can be accounted to florets, since the behaviour of green colour in broccoli stems could only poorly be described by the combined model.

The developed model makes it possible to pool data together in larger and therefore more reliable analyses. The description is generic in nature as can be taken from the common value for the kinetic parameters, irrespective of type of vegetable and location of measurement.

Table 12-5 List of Symbols

Variables	Meaning	Dimension
$a^*$	CIE-Lab $a^*$ value (green - red)	-
$ab$	ratio of $-a^*/b^*$	-
$b^*$	CIE-Lab $b^*$ value (blue - yellow)	-
$C$	Concentration green compounds	arbitrary
$E_a$	energy of activation	J/mol
$k$	rate constant	$\text{sec}^{-1}$
$L^*$	CIE-Lab $L^*$ value (lightness)	-
$N_{\text{obs}}$	number of observations	-
$R$	gas constant	8.314 J/K/mol
$R^2_{adj}$	percentage variance accounted for	%
s.e.	standard error of estimate	parameter dependent
stdev	standard deviation	-
$T$	temperature	$^{\circ}\text{C}$
$t$	time	s
Indices	Meaning	
0	initial, at time zero	
BF	for broccoli florets	
BS	for broccoli stems	
c	conversion	
d	decay	
fix	on invariable part	
GB4	for green beans April 1999	
GB8	for green beans August 1999	
i	running index	
max	of maximal colour development	
p	colour precursor	
ref	at reference temperature (60 $^{\circ}\text{C}$ )	

With the model, predictions can be made about the green colour dependence of temperature and time of the treatment. The temperature at which colour develops to maximal intensity of green can easily be deduced.

## Acknowledgement

Financial support by EU (grant nr. FAIR CT98-3155) is gratefully acknowledged.

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## **Part 5**

# **DISTRIBUTING QUALITY**

## Introduction

The main difference between normal logistics and logistics of agricultural produce is the continuous change in quality of agricultural products. The models of quality change for all the different kinds of fruits and vegetables in the assortment are considered too complex and too time consuming during simulation to be applied in models for logistics and distribution. Starting long before the age of computers and full scale modelling and simulation, efforts have been undertaken to develop simpler and more easy to use systems to get a grasp on the ever-changing quality of perishable produce on its way from grower to consumer. One of the more successful systems developed is that of shelf life (Sprenger 1937). The system is known under different names like e.g. shelf life, storage potential, quality life and keeping quality. Complete books with tables of shelf life values, that is how long can a certain product be kept with an acceptable level of quality, for a vast number of agricultural products in many different circumstances of storage and transport are available (e.g. Hardenburg et. al. 1986, Sprenger Institute 1986). The mathematical procedures and rules were advanced considerably (van Beek et al. 1985). Understanding of the basic fundamental problem, however, was minimal.

The system of keeping quality covers part of the psychological and economic effects, which affect the consumer in his decision whether or not to accept (to buy) a specific product or a specific batch of product. It is somewhere halfway between total neglect of consumer preferences, psychological and economic factors, and total coverage of them. Keeping quality expresses the time a product is considered acceptable for a majority of consumers in any situation of storage, transport and display. As a consequence of the definition, two main reasons exist for uncertainty in the keeping quality system: What is considered still acceptable and what constitutes a majority of consumers?

The keeping quality system does not take the actual level of quality present into account, but merely expresses the expected life span of a product. A consequence of full-scale application of keeping quality in chain optimisation is therefore that consumers eventually will be offered only produce that is just (barely) acceptable. With a temporary change (e.g. by fashion) in limit of acceptability, the whole optimisation collapses and the entire sector will witness a major drawback, like it happened in the nineties with greenhouse tomatoes produced in the Netherlands for export to Germany. So, the system of keeping quality is very useful, very powerful, very easily applied, but has its disadvantage.

Bringing together the principles of fundamental modelling with the practical system of shelf life determination, and providing some theoretical basis for that very successful system is the aim of this part. It was one of my earlier models developed that proved to be rather successful in application. After roughly 8 years of incubation, engineers more and more adopt its principles as a basis of a reliable and generic approach in dealing with quality in general (Schepers, A&F, personal communication) and with the problems of logistic in particular (Vollebregt, De Heij, A&F, personal communication).

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**13**

**A generic model for keeping quality  
of vegetable produce  
during storage and distribution.**

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Published in *Agricultural Systems* 1996, **51**, 431-452.

Updated according to: Hertog M.L.A.T.M., Tijskens L.M.M. (1998). Modelling Modified Atmosphere Packaging of perishable produce: keeping quality at dynamic conditions. *Acta Alimentaria* **27**, 53-62.

## Abstract

A generic model on the keeping quality of perishable produce was formulated, based on the kinetics of the decrease of individual quality attributes. The model includes the effects of temperature, chilling injury and different levels of initial quality and of quality acceptance limits. Keeping quality of perishable produce was found to be inversely proportional to the sum of the rates of the separate reactions leading to quality decrease, irrespective of the kinetics of the decrease.

In its static form, the model is useful for statistical analysis and for predicting keeping quality at constant conditions. In its dynamic form, it predicts keeping quality as a function of temperature, initial quality and quality acceptance limits. These limits are defined by personal, regional or national preferences. Calculation of the dynamic model requires only one simple numerical integral, even for multiple limiting attributes. Due to the fast numerical integration of that one integral, optimisation of distribution chains with respect to produce quality over a broad time and space region becomes economically feasible.

The model accounts for the behaviour of keeping quality of about 60 species of fruits and vegetables, including chilling sensitive products, over a wide range of temperatures.

## Introduction

Whether or not a product is acceptable to the user depends on the product quality and on the level of the acceptance limit (Tijsskens et al. 1994c). The limit of acceptance is largely defined by the economical and psycho-social circumstances of the user; the quality of a product is largely defined by its intrinsic properties. As a consequence, product acceptability will partly depend on product behaviour and partly on consumer attitude. The concept of keeping quality combines both these aspects of product acceptance, that is, acceptance limit and intrinsic properties, into a generally applicable and simplified index of quality.

In order to describe, analyse and predict the response of quality to various constant and varying circumstances, the problem has to be decomposed. The underlying processes can be categorized as objective (e.g. chemical, physical, physiological processes) or as subjective (e.g. sensory perception, evaluation and acceptance). Much research concerning quality of foods is devoted to the search of those objective properties on which the subjective perception and evaluation of specific quality attributes are based (Watada et al. 1984, Janse 1989, Shewfelt 1990, Polderdijk et al. 1993, Reid 1992, Kader 1992).

The keeping quality of a product has frequently been used as a simple, attribute-unspecific identifier of overall quality (Sprenger 1937, Hardenburg et al. 1986, van Doorn & Tijsskens 1991, Polderdijk et al. 1993). It has also been used to describe the relation of product quality with bacterial infection and growth (Labuza et al. 1992, Fu & Labuza 1993, Willocx et al. 1993) and with technical application of cooling (Meffert & van Vliet 1974, Segurajaurgui Alvarez & Thorne 1981, van Beek et al. 1985).

With internal quality becoming increasingly important and with internal quality becoming an explicit part of supermarket policy (Monnot 1990), application of a reliable model on internal quality is necessary. To simplify the observed phenomena into an acceptable descriptive model, it is essential to break down the complexity of the various processes that affect quality, otherwise the simplification may be arbitrary, resulting in unreliable model formulations. The present paper relates overall keeping quality to the kinetics of the decrease of its individual quality attributes. The effect of temperature on keeping quality has been described independent of kinetic type. Information about the type of kinetic mechanism is only needed if the initial quality and the quality limit are different from the measuring conditions.

## Definition of Keeping Quality

A generally accepted definition of keeping quality is the time until a commodity becomes unacceptable (van Beek et al. 1985, Fu et al. 1993). The attribute limiting the product acceptance can be predefined (e.g. firmness) or may depend on circumstances (e.g. it can

be firmness or colour whichever attribute first becomes unacceptable). Keeping quality only provides information about the time the product can be kept prior to becoming unacceptable, but it does not provide information either about the actual state of the product's quality or about the processes occurring in the product. Without information, however, about the mechanisms involved in the decrease in quality or quality attributes, the dynamics of keeping quality can not be described.

A new approach will be developed relying on the kinetics of the possible mechanisms involved, starting from the most simple situation of a single quality attribute in a constant environment, to several quality attributes in a constant environment, and finally towards complex quality attributes in a varying environment. Taoukis & Labuza (1989) have already described this approach for a single quality attribute only, solving the problem with a physical (diffusion) approach, without fully exploring the chemical approach.

## Methods

The equations in the mathematical description of the model have been developed and solved using MAPLE V (Waterloo Maple Software, Waterloo, Canada), a computer algebra program for manipulation of symbolic functions.

The data used in the statistical analysis have been read back from published graphs of keeping quality versus storage temperature for a number of products (Sprenger Institute 1986, Paull 1993). Data on the keeping quality of sweet basil in relation to diurnal harvest time have kindly been provided by Lange & Cameron (1994). The statistical analysis was carried out using nonlinear regression (GENSTAT Statistical Package, Rothamstead, UK). To avoid the introduction of unnecessary errors by transformation of data (Ross 1990, Tijsskens 1994) no transformation was applied.

The dynamic model is implemented in PROSIM (Sierenberg & de Gans, Waddinxveen, The Netherlands), a modelling language which combines the benefits of continuous, discrete, mixed and parallel modelling.

## Mathematical Description of the Model

### ***Single limiting quality attributes at constant temperatures***

For simple situations in constant storage conditions the possibly complex behaviour of quality will reduce to a rather simple behaviour: by fixing the one storage temperature, inevitably, for a given value of initial quality and of quality limit, the attribute first to become unacceptable will always be the same. So, in a constant environment, the behaviour of only one quality attribute has to be considered in the deduction of the equations. What is not yet defined is the mechanism involved, or the (chemical) path along which the change of that particular quality attribute will take place. In practice the decrease of a single quality attribute can be approximated by one of the four following basic types of mechanism (Taoukis & Labuza 1989):

- zero order reactions having linear kinetics
- Michaelis Menten kinetics
- first order reactions having exponential kinetics
- autocatalytic reactions with logistic kinetics.

For each of these types a relation between keeping quality and the underlying reaction mechanism will be deduced. It has to be pointed out that the only assumptions made in coming deduction of the equations, lies in the mechanism chosen for a particular quality attribute. The deduction of the equations themselves is conducted entirely according the fundamental laws of kinetics. Figure 13-1 gives a summary of the behaviour of quality attributes based on these four kinetic mechanisms. Keeping quality is the time until the quality crosses the acceptance limit. With the same initial quality and the same quality limit, Figure 13-1 shows the effect of the kinetic mechanisms on keeping quality as depending on the level of the quality present and on the level of the acceptance limit.

### *Linear and Michaelis Menten kinetics*

Although linear kinetics or zero order reactions are relatively rare, Michaelis Menten kinetics are observed more frequently. This type of kinetics reduces to a linear one if the amount of substrate (here quality attribute) exceeds the specificity factor  $K_m$ , which is most likely the case in the initial region of decay. This initial part is the most important in quality assessment (see Figure 13-1). If it should not be the case, Michaelis Menten kinetics reduce to the exponential type, covered in the next section.

For a quality decrease according to a zero order reaction, the following kinetics can be derived (variables are defined in Table 13-6 Nomenclature):

$$\frac{dQ}{dt} = -k \quad \text{eq. 1}$$

Integration at constant temperature results in a linear relation:

$$Q - Q_0 = -k \cdot t \quad \text{eq. 2}$$

where  $Q_0$  is the initial value of  $Q$ .

When the quality  $Q$  exceeds the quality limit  $Q_l$ , the time elapsed is equal to the keeping quality, hence:

$$KQ = \frac{Q_0 - Q_l}{k} \quad \text{eq. 3}$$

The keeping quality  $KQ$  for zero order kinetics is proportional to the inverse of the reaction rate constant  $k$  of the decrease in quality.

#### Exponential kinetics

First order reactions leading to exponential responses are commonly encountered in natural processes. Based on fundamental kinetics, the relevant differential equation is:

$$\frac{dQ}{dt} = -k \cdot Q \quad \text{eq. 4}$$

Assuming constant temperatures, integration gives:

$$Q = Q_0 \cdot e^{-k \cdot t} \quad \text{eq. 5}$$

From this relation, the keeping quality can again be derived as the time at which the quality  $Q$  reaches the quality limit  $Q_l$ :

$$KQ = \frac{\log_e \left( \frac{Q_0}{Q_l} \right)}{k} \quad \text{eq. 6}$$

Again, as for linear decay, the keeping quality for exponential decay is proportional to the inverse of the rate  $k$  of quality decrease.

#### Logistic kinetics

Logistic behaviour is also frequently encountered in natural processes (France & Thornley 1984 pp. 75, Thornley 1976 p. 10, Tijssens et al. 1994a, 1994b). Logistic behaviour can be

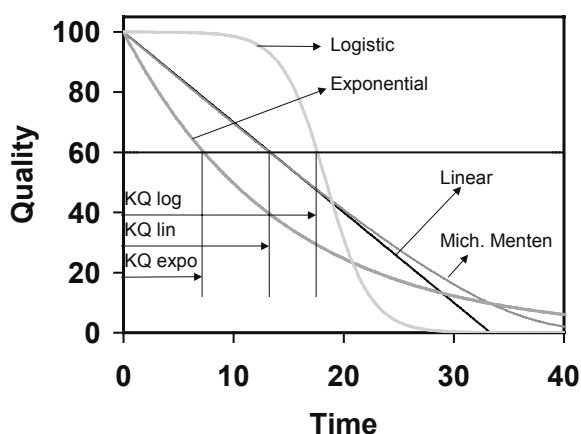


Figure 13-1 Decrease in quality for several types of mechanisms (arbitrary units).

regarded as the overall expression for autocatalytic processes, diffusion controlled processes, cascades of reactions and complex growth kinetics. The formulation of this type of reactions can be written in different forms. One is shown in equation 7:

$$\frac{dQ}{dt} = -k \cdot Q \cdot \left( \frac{1-Q}{Q_{\text{inf}}} \right) \quad \text{eq. 7}$$

Again by integration assuming constant temperatures, one obtains:

$$Q = \frac{Q_{\text{inf}}}{1 - C_{\text{ba}} \cdot e^{k \cdot t}} \quad \text{eq. 8}$$

with  $C_{\text{ba}} = \frac{Q_{\text{inf}} - Q_0}{Q_0}$

From this relation, the keeping quality can again be derived as the time at which the quality  $Q$  reaches the quality limit  $Q_l$ :

$$KQ = \frac{\log_e \left( \frac{Q_{\text{inf}} - Q_l}{Q_l \cdot C_{\text{ba}}} \right)}{k} \quad \text{eq. 9}$$

$Q_{\text{inf}}$  represents the quality maximally possible at (minus) infinite time, while  $C_{\text{ba}}$  is a constant representing information about the biological age of the product (Tijskens et al. 1994a). Again the keeping quality is inversely proportional with the rate  $k$  of quality decrease.

#### *Effect of temperature*

From the above discussion it follows that keeping quality is proportional to the inverse of the reaction rate constant  $k$  of the decrease in quality, irrespective of the kinetic mechanism of the decrease. This gives the opportunity to describe the behaviour of keeping quality as a function of temperature. Specific rates of chemical, biochemical and enzymatic reactions usually depend on temperature according to Arrhenius' law (Chang 1981). Apparent rates, e.g. of enzymatic reactions, can at the relatively low storage temperatures encountered in practice, also be approximated by this law:

$$k = k_{\text{ref}} \cdot e^{\frac{E_a}{R} \left( \frac{1}{T_{\text{ref}}} - \frac{1}{T_{\text{abs}}} \right)} \quad \text{eq. 10}$$

The energy of activation  $E_a$  has a positive value, indicating an increase in reaction rate constant with increasing temperature. Due to the inverse relation with the reaction rate constant  $k$  the keeping quality will also depend on temperature according to Arrhenius' law. Application of the Arrhenius equation for the temperature dependence of the reaction rate constant  $k$  of quality decrease is in accordance with Taoukis & Labuza (1989). Segurajaurgui Alvarez & Thorne (1981) reported an empirical exponential relation between keeping quality and temperature.

#### *Influence of initial quality and quality limits*

The keeping quality in a constant environment can be represented as:

$$KQ = \frac{f(Q)}{k} \quad \text{eq. 11}$$

where  $f(Q)$  is an expression comprising the initial quality  $Q_0$  and the limiting quality  $Q_l$ . The exact formulation of  $f(Q)$  depends on the reaction kinetics governing the decrease of the limiting quality attribute. In Table 13-1 an overview is given for the respective quality functions.

Taoukis & Labuza (1989) found the same relation with kinetic mechanism and called the factor  $f(Q)$  the quality function. Equation states that a generic formulation of keeping quality

can be developed, irrespective of the reaction mechanism involved. Within a situation constant for  $Q_0$ ,  $Q_l$ , and for other quality references that mechanism dependent (see Table 13-1), the quality function is constant. Consequently, keeping quality does not depend on the type of reaction kinetics.

The limit of acceptance is of major importance in the concept of keeping quality. It can be defined as the minimal quality necessary for a consumer to accept the product (Tijskens et al. 1994c). When the initial quality is different from the initial quality of the measuring batch of products or when the quality limit changes during storage or distribution, the kinetic mechanism will, however, exert a strong effect on the observed behaviour of keeping quality by changing the value of the quality function  $f(Q)$ . Equation 11, combined with the quality function shown in Table 13-1, how and when the initial quality and the setting of the quality limit will affect the keeping quality. The effect of both parameters can be visualised by imagining the quality limit to shift over the Y-axis or the initial quality to change by a shift of the imaginary starting point of the experiment over the X-axis. Keeping quality is then, by definition, the time at which the quality crosses the quality limit line. The change in keeping quality depends strongly on the kinetics of the quality decrease in time.

Table 13-1 Overview respective quality functions

Kinetic mechanism	$f(Q)$
linear	$Q_0 - Q_l$
exponential	$\log_e \left( \frac{Q_0}{Q_l} \right)$
logistic	$\log_e \left( \frac{Q_{inf} - Q_l}{Q_l \cdot C_{ba}} \right)$

### **Multiple limiting quality attributes at constant temperatures**

In many horticultural products, the quality attribute that limits the acceptance by the consumer shifts from one attribute at a certain temperature to another attribute at another temperature. This can for example be observed in chilling sensitive products. For the description of this more complex situation, let us assume that the storage temperature remains constant during the whole storage period, but the quality attribute that limits the shelf life of the product, changes from one attribute to another depending on the level of the constant temperature. In tomatoes for example kept at constant temperatures below 8 °C the limiting factor is usually the colour, above 13 °C it is firmness. The extension to be made to the previous model is to determine which quality attribute is limiting at which temperature. Each separate quality attribute has to be described by its own kinetic mechanism. Next, all the quality attributes described have to be combined in some expression. The central issue in this problem is to deduce that expression for combining the effects of the separate quality attributes.

In solving this problem a distinction has to be made between non-interfering and interfering quality attributes. Non-interfering attributes can be considered as additive at the level of differential equations, interfering ones as multiplicative.

#### *Non-interfering processes*

In non-interfering processes, the change of each quality attribute can be described in its own right without interference of the other quality attributes. So, part of the overall quality decrease is connected to that specific process. The combination of each of these processes for each of the quality attributes then describes the decrease of overall quality. Unless explicitly asked for, consumers and expert panellist judge directly overall quality without asserting the different attributes separately and combining them into a final judgement. Assuming the same type of kinetics for each attribute (e.g. first order), this situation for three separate processes, acting on the same overall quality, is depicted in equation 12. For the three different processes, each potentially effective in decreasing quality, one could consider e.g. chilling injury (index 1), decrease of quality at intermediate temperature (index 2) and high temperature injury (index 3).

$$\begin{aligned}
Q &\xrightarrow{k_1} \text{decrease} \\
Q &\xrightarrow{k_2} \text{decrease} \\
Q &\xrightarrow{k_3} \text{decrease}
\end{aligned}
\tag{eq. 12}$$

$$\text{hence } \frac{dQ}{dt} = -(k_1 + k_2 + k_3) \cdot Q$$

Solving the differential equation for overall quality at constant temperatures generates a solution as shown in equation 13, which is very similar to the solution for a single limiting attribute (eq. 6):

$$KQ = \frac{\log_e \left( \frac{Q_0}{Q_1} \right)}{k_1 + k_2 + k_3}
\tag{eq. 13}$$

Each of the individual reaction rate constants, however, will exhibit its own Arrhenius type dependency on temperature (see eq. 10). Consequently, the keeping quality will be inversely proportional to the sum of the three rate constants, each with their own temperature relation. The process or quality attribute that limits the acceptance of the product at a particular temperature will be the dominant factor in the denominator at that temperature, thereby effectively reducing the influence of the other two processes at that temperature. The level of constant temperature will determine which one of the three processes is limiting.

Of course, the formulation will be different for the other types of kinetics (linear or autocatalytic). That situation is not worked out in this paper. The solutions are, however, similar to the respective single attribute situation but again with a summation over all the reaction rate constants in the denominator. The situation will also be different and much more complicated if the type of kinetics is not the same for the three processes. One of the processes, however, will prevail at a certain temperature, thereby determining for the major part the mechanism involved.

#### *Interfering processes*

When the processes or quality attributes do interfere with one another, the situation becomes very complex. Logical assumptions for obtaining a common or generic model can no longer be made. In that case, the different processes, including the interferences, have to be modelled separately. The formulation and implementation of interfering processes are well beyond the scope of this study. An example for this approach can be found in the chilling injury model derived by Tijskens et al. (1994b).

## Results and Statistical Analysis

Both systems, a single limiting attribute for a range of temperatures, and multiple limiting attributes shifting gradually over the temperature range, can be described in one function relating keeping quality with temperature. In the equations 3, 6, 9 and 10 no algebraic simplifications were applied, nor was it verified whether each parameter could be statistically estimated. From the four parameters in equation 13 (one on the numerator and three in the denominator) only three can be estimated: the equation is over-parameterised. In equation 14, the system is fully developed towards a formulation more useful for practical application and statistics.

$$KQ = \frac{KQ_{\text{ref}}}{\sum_{i=1}^N k_{\text{ref}}(i) \cdot e^{\frac{Ea(i)}{R} \left( \frac{1}{T_{\text{ref}}} - \frac{1}{T_{\text{abs}}} \right)}}
\tag{eq. 14}$$

Table 13-2 Estimated parameters for a single limiting attribute, based on equation 14. Data from Sprenger Institute (1986).

Product			Estimated parameters			Standard Error	
	$R^2_{adj}$	N	$KQ_{ref}$	$k_{ref}(1)$	$Ea(1)$	$KQ_{ref}$	$Ea(1)$
Asparagus spears	98	1	2.876	1	108.23	0.359	9.85
Bean broad	95	1	3.701	1	104.09	0.575	11.81
Blackberry	99	1	2.768	1	55.45	0.073	2.30
Brussels Sprouts	94	1	1.552	1	142.56	0.408	17.19
Cabbage, Chinese	100	1	6.814	1	81.62	0.223	2.45
Cabbage, Savoy green	95	1	0.657	1	275.56	0.385	32.55
Cabbage, Savoy red	92	1	12.250	1	168.52	4.710	27.07
Cabbage, Savoy yellow	92	1	12.250	1	168.52	4.710	27.07
Cabbage, White	93	1	20.150	1	154.35	6.380	22.56
Carrot, winter	93	1	25.190	1	118.33	6.180	18.16
Carrot, unwashed	98	1	8.401	1	200.17	1.800	15.21
Carrot, washed	95	1	1.983	1	165.07	0.465	17.18
Cauliflower	99	1	6.074	1	124.24	0.461	5.55
Celeriac	99	1	35.460	1	98.54	1.620	3.55
Celery	100	1	4.920	1	112.39	0.297	4.01
Celery, blanched	94	1	0.622	1	240.08	0.343	37.81
Cherry	100	1	4.913	1	73.14	0.093	1.70
Chicory	99	1	5.347	1	93.81	0.221	3.18
Currant, black	98	1	3.096	1	85.67	0.232	5.18
Currant, red	99	1	5.001	1	86.68	0.291	4.61
Endive	98	1	2.914	1	99.08	0.381	9.73
Gherkin	95	1	6.602	1	91.01	0.548	11.21
Gooseberry, ripe	99	1	5.001	1	86.68	0.291	4.61
Gooseberry, unripe	99	1	7.869	1	79.35	0.403	4.12
Grape	100	1	33.500	1	113.89	1.230	2.83
Kale	97	1	3.892	1	122.08	0.601	11.65
Kohlrabi + leaf	100	1	12.680	1	57.31	0.092	0.58
Leek	98	1	5.311	1	116.57	0.865	10.51
Lettuce	99	1	2.762	1	91.58	0.233	6.36
Lettuce Iceberg	100	1	4.885	1	68.11	0.135	2.16
Mushroom	100	1	2.410	1	51.50	0.031	1.16
Onion	99	1	99.570	1	55.67	5.140	3.35
Onion, cut	99	1	1.825	1	34.94	0.044	1.90
Onion, hand peeled	94	1	4.499	1	73.38	0.600	10.21
Onion, mach. dry peeled	100	1	3.374	1	49.82	0.021	0.52
Parsley	100	1	5.099	1	100.81	0.242	3.30
Pea green	99	1	2.194	1	72.36	0.084	3.25
Peach	88	1	2.105	1	110.58	0.503	18.09
Plum Victoria	99	1	6.393	1	71.78	0.412	4.91
Purslane	97	1	2.586	1	47.46	0.119	4.11
Radish, Black + leaf	98	1	2.276	1	77.43	0.109	3.94
Radish, Black – leaf	100	1	24.730	1	102.03	1.090	3.41
Raspberry	97	1	2.528	1	49.85	0.133	4.64
Rhubarb + leaf	98	1	2.144	1	81.23	0.107	4.04
Rhubarb – leaf	100	1	6.221	1	75.66	0.051	0.68
Spinach	100	1	2.523	1	72.88	0.071	2.22
Strawberry	100	1	2.226	1	37.35	0.010	0.41
Turnip	99	1	62.910	1	68.88	2.690	3.36



N stands for the number processes contributing to the keeping quality. This number is usually not greater than two. The value of the sum of the reaction rate constant at reference temperature is put to one, to avoid overparametrisation. So, the numerator  $KQ_{ref}$  in equation

14 stands for the quality function divided by the sum of the reaction rate constants  $\frac{f(Q)}{\sum_{i=1}^N k_{ref}(i)}$ .

$f(Q)$  is called the quality function by Taoukis et al. (1989) and is defined by the type of kinetics involved (see eq. 3, 6, 9 and Table 13-1). The reference reaction rate constants in the denominator each represent the relative importance of the  $N^{\text{th}}$  quality process at the reference temperature. For applications in practice the process active in decreasing the quality and the quality attribute it is connected to, can be considered exchangeable, as they will be linked together in most of the cases on a one-to-one basis.

In both the single and the multiple process system  $KQ_{ref}$  represents the keeping quality at the reference temperature. At the same reference temperature,  $KQ_{ref}$  can be directly used to compare the keeping quality of various products. By putting the sum of the reference reaction rate constants to one, not only statistical analysis becomes possible without information of the level of the quality function  $f(Q)$ , but the expression for keeping quality become virtually independent of the kinetic mechanism involved.

The hypothesis that keeping quality is inversely proportional to the sum of the reaction rate constants of the decrease in quality, irrespective of the mechanism and the number of limiting attributes involved, has been tested on data for a number of products (Sprenger Institute 1986, Paull 1993). The reference temperature is arbitrarily chosen to be 10 °C. First the data were analysed with N equal 1. When the percentage variance accounted for ( $R^2_{adj}$ ) was smaller than 90%, for example for chilling sensitive products, N was put to two and the data were reanalysed estimating the both reaction rate constants and both activation energies, forcing their sum to be equal to one. In Table 13-2 the results and standard errors for about 50 products with only a single limiting attribute are shown, Table 13-3 shows the

Table 13-3 Estimated parameters for two limiting attributes, based on equation 14. Data from Sprenger Institute (1986).

	$R^2_{adj}$	N	Estimated parameters				
			$KQ_{ref}$	$k_{ref}(1)$	$k_{ref}(2)$	Ea(1)	Ea(2)
Bean, French	80.0	2	5.674	0.9480	0.0520	80.74	-271.80
Bean, runner	92.5	2	5.721	0.9986	0.0014	92.90	-479.32
Bean, slicing	85.1	2	4.848	0.9639	0.0361	72.46	-283.79
Beetroot	99.1	2	72.322	0.9548	0.0452	175.65	-157.82
Bell pepper	98.6	2	11.086	0.9391	0.0609	128.55	-336.19
Cucumber	97.6	2	8.953	0.5953	0.4047	36.10	-159.69
Kohlrabi –leaf	98.9	2	40.795	0.9998	0.0002	107.49	-411.05
Papaya*	95.5	2	24.816	0.6523	0.3477	53.10	-177.83
Tomato	98.6	2	6.389	0.2409	0.7591	77.91	-421.38
			Standard Error				
Bean, French			0.5086	0.0658		26.46	100.21
Bean, runner			0.6026	0.0028		20.54	148.11
Bean, slicing			0.2880	0.0381		20.69	81.84
Beetroot			3.8182	0.0567		28.47	98.16
Bell pepper			0.2876	0.0179		9.37	29.03
Cucumber			0.3543	0.0993		7.98	25.54
Kohlrabi –leaf			2.1612	0.0000		14.48	355.35
Papaya*			0.9739	0.0792		6.46	23.68
Tomato			0.4557	0.0422		13.26	59.04

\*data R. Paull, 1993

results for products with two limiting attributes.

## Further Development and Validation

In the formulation of the model (eq. 14) the effects of initial ( $Q_0$ ) and boundary conditions ( $Q_i$ ) are separated from the dynamic processes ( $k(i)$ ). As a consequence, all terms and parameters have a distinct physical or chemical meaning. The external storage conditions like temperature, controlled atmosphere, modified atmosphere and relative humidity, will have only an effect on the rate of the occurring reactions. Preharvest or growth conditions will have only an effect on the potential keeping quality ( $KQ_{ref}$ ) and will act upon the level of initial quality. Based on the data of Lange et al. (1994) on the keeping quality of sweet basil as affected by the diurnal harvest time, this aspect of the formulation for keeping quality was investigated. It was found that for each of the harvest times (from 2 a.m. to 10 p.m.) the reaction rate constants and their temperature dependency were almost the same. Only the potential keeping quality ( $KQ_{ref}$ ) was affected by the diurnal harvest time, showing a sinusoidal behaviour with harvest time. The applied formulation for keeping quality was altered to include this phenomenon:

$$KQ = \frac{KQ_{max} \cdot (\beta + \sin(\omega \cdot Harvesttime + \alpha))}{\sum_{i=1}^N k(i)} \quad \text{eq. 15}$$

Nonlinear regression on the complete set of keeping quality data with diurnal harvest time, time and temperature of subsequent storage as explaining variables gave a percentage variance accounted for ( $R^2_{adj}$ ) of about 90%. Omega is fixed to the 24 hr frequency ( $2 \cdot \pi / 24$ ). The estimated parameters and their standard error are shown in Table 13-4. The difference between measured and calculated keeping quality was well within the range of observed variation.

Table 13-4 Estimated parameters for Sweet Basil according equation 15.  
Data from Lange et al. 1994

	$R^2_{adj}$	$KQ_{max}$	$k_{ref}(1)$	$E(1)$	$k_{ref}(2)$	$E(2)$	$\alpha$	$\beta$
Parameter	89.3	1.796	0.2567	79.5	0.731	-124.5	3.340	5.22
Stand error		0.357	0.0885	16.2		19.9	0.194	1.02

## Discussion

The model was found to be generic and applicable to all products analysed, from moderate as well as from tropical areas. The percentage variance accounted for ( $R^2_{adj}$ ) is high and all standard errors are relatively small, irrespective number of attributes involved, irrespective of the absolute magnitude of the keeping quality and irrespective of the origin of the commodities (from tropical or moderate areas). In the analysis of 60 commodities only three products showed a percentage variance accounted for ( $R^2_{adj}$ ) below 90%. Increasing the number of attributes to three or more was never required. Statistical validation for each commodity separately ( $R^2_{adj}$ ) combined with generic validation (number of products) indicates the model to be quite reliable and powerful. In Figure 13-2 the measured and calculated keeping quality for a single ( $N=1$ ,

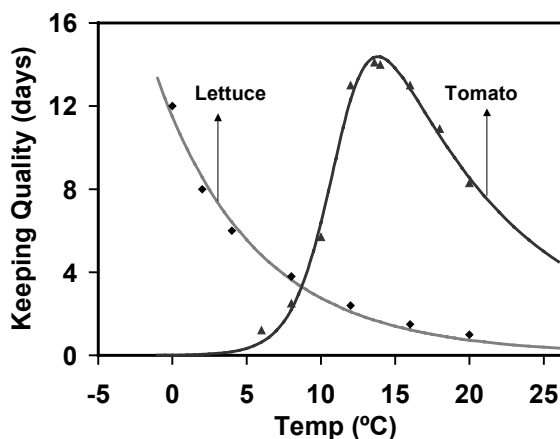


Figure 13-2 Measured (points) and calculated (solid line) keeping quality for lettuce and tomato.

lettuce) and a double ( $N=2$ , tomato) attribute system is shown.

Some products exhibit a value for keeping quality at higher temperatures, somewhat higher than predicted by the model (see Figure 13-3 for Brussels sprouts). This deviation could not be fixed by introducing of another term ( $N>1$ ). Apparently, some threshold in keeping quality is present for these types of products. This may be due to a mix of types of mechanisms in equation 12, e.g. one exponential with one linear mechanism (see section Non-interfering processes). This mathematical system with mixed mechanisms has been solved using MAPLE V. The solution, however, was not suitable for a generic approach, and was excluded from further investigation.

External factors (like temperature and harvest time) behave indeed as postulated in the deduction of the formulation. As a consequence, the applied decomposition of the problem and the resulting model can be considered to be valid. What causes the sinusoidal effect of the diurnal harvest time can not be discovered with the available data. A link to the intensity ( $\alpha$ ) and the quality ( $\beta$ ) of the sunlight seems to be likely.

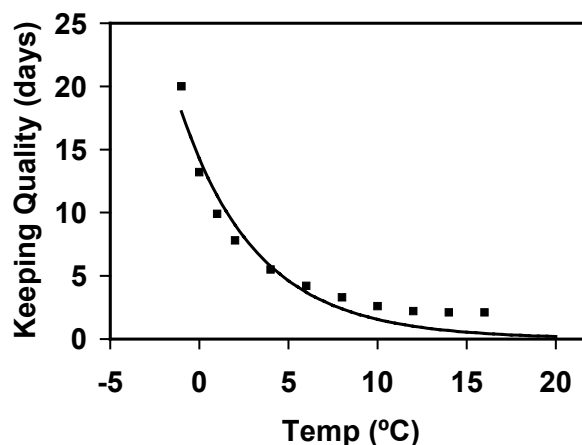


Figure 13-3 Measured (points) and calculated (solid line) keeping quality for Brussels sprouts, showing aberration at higher temperatures.

## Dynamic Model

Until now, the model describes the keeping quality at fixed storage temperatures. Temperatures, however, usually change dynamically during the lifetime of a commodity. The fact that the proposed model fits well for a large number of products over a broad range of constant temperatures indicates that in conditions occurring in practice, the basic assumptions are valid. If these assumptions are valid and if the quality attribute, that limits the keeping quality at a certain temperature, changes gradually from one attribute to another with changing temperature, then a dynamic approach is allowed. As keeping quality is not a fundamental property like quality or a quality attribute, but a secondary one, predicting the time (to come) the product can endure, keeping quality itself can not be modelled dynamically. Based on the derivation of the proposed model, however, the necessary dynamics can easily be deduced from the kinetics for the overall quality (eq. 1 to 9).

### **Constant boundary conditions**

With a dynamically changing temperature acting on a decreasing quality, the remaining keeping quality at some standard temperature has to be calculated to compare different time temperature combinations and scenarios. This is the same technique as used by Labuza et al. (1992), Sprenger Institute (1986), van Beek et al. (1985) and Paull (1993). The remaining keeping quality is then called shelf life. The standard temperature may be chosen as appropriate for a particular application or commodity. To compare various commodities, however, application of the same standard temperature is recommended.

The quality function  $f(Q)$  for each type of kinetics is exactly the inverse function of the quality behaviour at constant temperature. Consequently, the keeping quality will change linearly during the (very small) time period during which the temperature can be considered constant. For each day of storage a certain fraction of keeping quality will vanish. The slope of the linear change will depend on the storage temperature as described by the complex rate constant (see the denominator of eq. 14). Provided the quality limit remains the same throughout the storage period and provided the initial quality is the same as or comparable to the measuring situation, the dynamic model can be formulated as:

$$KQ_{st} = \frac{KQ_{ref} - \int_0^t \sum_{i=1}^N k(T(t)) \cdot dt}{\sum_{i=1}^N k(T_{st})} \quad \text{eq. 16}$$

Although the type of kinetic mechanism of quality decrease is of major importance for the quality attribute itself, it completely disappears from the equation for keeping quality by the mere fact that the quality function  $f(Q)$  is the inverse function of quality decay. If the model on keeping quality is solely applied on a local level (that is a constant limit of quality acceptance), or if one is only interested in keeping quality for reason of comparison (e.g. chain optimisation) keeping quality can dynamically be estimated without any information about the type of mechanism involved.

**Variable boundary conditions**

For the description and application of dynamic keeping quality when the initial quality and/or the quality limit do not remain constant the situation is more complicated. The value of the quality attribute, determining the consumer acceptance, can no longer be represented by its analytical function but has to be expressed as the integral of the change in time with varying environmental temperatures. This dynamic change in quality applies the direct reaction rate constants instead of the relative ones with their sum equal one as used in equation 14 and estimated in Table 13-2 and Table 13-3. These direct reactions rates can be calculated if the initial quality ( $Q_{m0}$ ) and quality limit ( $Q_{ml}$ ) of the measured data set are known. The ratio between measuring conditions and actual conditions provides the necessary correction factor. The dynamic keeping quality with variable initial and limiting quality can be formulated as in equation 17.

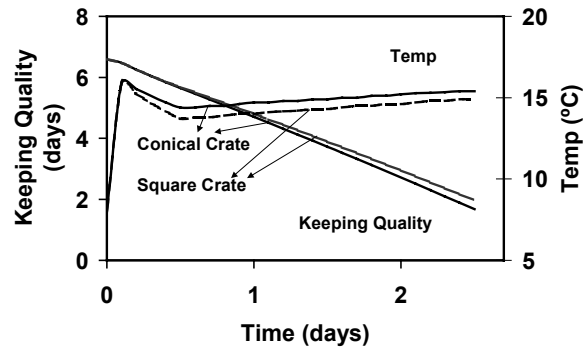


Figure 13-4 Dynamic keeping quality of French beans at different temperature histories in two different crate symmetries.

$$KQ_{dyn} = \frac{\log_e \left( \frac{Q_{b0} - \int_{t=0}^t \frac{-\log_e \left( \frac{Q_{m0}}{Q_{ml}} \right) \cdot k(T(t)) \cdot Q_b(t) \cdot dt}{KQ_{ref}}}{Q_{bl}} \right) \cdot KQ_{ref}}{\log_e \left( \frac{Q_{m0}}{Q_{ml}} \right) \cdot k(T_{st})} \quad \text{eq. 17}$$

This equation not only corrects dynamically for varying initial quality, but allows also discrete changes in quality limit.

The possibilities and benefits of application of the dynamic model (eq. 17) in international transport have already been presented (Tijskens et al. 1994d). The dynamic keeping quality formulated in equation 16 has successfully been used in visualising differences in temperature profiles over different type of packages. In a simulated transport two designs of storage crates generated for the same sequence in external temperature, shown in Table 13-5, a slightly different sequence of product temperature. Based on this product

temperature sequence, the keeping quality of stored French beans was calculated according to equation 16. In Figure 13-4 the calculated keeping quality is shown for French beans in the two dynamically changing product temperature scenarios.

## Conclusions

Keeping quality can be modelled by one generic model suitable for static applications and for statistical analysis. More than 60 different horticultural products comply in storage at constant temperature to the proposed model with a high degree of reliability.

Dynamic modelling of keeping quality is possible provided the dynamic changes in temperature are not too excessive over the storage and distribution period.

The mechanism of quality decrease, most often neither recorded nor reported, provides the necessary information to allow describing the influence of initial and limiting quality. Based on this knowledge and on the derived relations, changes in keeping quality by differences between international and local acceptance (for example fashion and preferences) can be described and predicted. Consequently, information based on local acceptance can be translated to regions with different accepting customs.

The applied decomposition of the problem allows one to link the effects of external factors, preharvest and postharvest, to those model parameters that, based on the common rules of kinetics, are most likely to be involved.

Optimisation, with respect to quality, of complete storage and distribution chains now comes into perspective.

Table 13-5 Temperature sequence in simulated transport.

Action	Temp ( C)	Time (hr)
From Auction to Export Firm		
Transport	8	1
Packing	16	1
Storage	8	4
From Export Firm to Distribution Centre		
Transport	8	4
Storage	8	2
From Distribution Centre to Retail		
Transport	19	2
Sales	19	48

Table 13-6 Nomenclature

Variable	Dimension	Description	Indices	
C	[-]	correction for biological age	abs	absolute (temperature)
Ea	[kJ/mol]	energy of activation	b	a particular batch
f()	[-]	unspecified function	ba	biological age
harvesttime	[hr]	diurnal time of harvest	inf	at (minus) infinite time
k	[ <sup>2</sup> ]	quality reaction rate constant	l	limit
KQ	[time]	keeping quality	m	the measuring batch
N	[-]	number of processes	max	maximal
Q	[qau <sup>1</sup> ]	quality (amount, intensity, level etc)	ref	at reference temperature
R	[J/mol/K]	gas constant = 8.314 J/mol/K	st	at standard temperature
t	[time]	time	0	initial
T	[K or °C]	temperature	1,2,3	process indication
a	[-]	diurnal time shift factor		
β	[-]	translation factor		
ω	[hr <sup>-1</sup> ]	daily frequency = 2*π/(24 hr)		
<sup>1</sup> Quality arbitrary unit: the dimension of quality is rather arbitrary, and depends on type of measurement and type of attribute measured. As long as one keeps the dimensions consistent, no problems arise.				
<sup>2</sup> The dimension of reaction rate constant depends on the mechanism of the reaction: time <sup>-1</sup> for exponential and logistics mechanisms qau*time <sup>-1</sup> for linear mechanism				

Both fundamental as well as applied research can use the same concept of keeping quality, based on the simplified description of quality decay. This will greatly enhance the exchange of information between fundamental research and practical application.

## Acknowledgement

*This study was conducted in the framework of a research program on fruits and vegetables, partly financed by the Dutch Commodity Board for Vegetables and Fruits. The valuable discussions with Dr. W. G. van Doorn and Prof. Dr. J. de Baerdemaeker are gratefully acknowledged.*

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## **Part 6**

### **VARIANCE OF QUALITY**

## Introduction

As already pointed out in the first part on modelling, the processes that nature uses are basically simple. The complexity of observations in nature comes from the multitude of interactions and combinations that nature applies based on these simple processes. Based on only four nucleotides genes are built up in an almost infinite number of sequences. Chromosomes are also very limited in number (around 40 in mammals) while no single individual is or was ever the same as another individual. When humans, and scientists for that matter, encounter in nature phenomena beyond contemporary understanding, they simply call them biological variance.

The complete system for food production, handling and distribution is entirely based on as homogenous batches as possible. The large machinery, involved in the food supply chain, can simply not handle that kind of differences. To avoid as much as possible the danger of not meeting the expectations of buyers and consumers refuge is sought in sorting and grading. All kind of intelligent techniques and procedures for grading and sorting on appearance, size and defects have been developed and are used on a daily basis, just to avoid the burden of biological variance.

With the increasing importance of produce grown all over the world and transported around the globe, the variation between batches will increase quite drastically. At the same time, consumers put more and more emphasis on internal quality of their food. Grading systems on internal quality attributes like taste, flavour, or contents, however, are very scarce. As a consequence, the entire food chain, the research on agricultural commodities not excluded, will increasingly be confronted with differences between batches from different regions and different seasons, which cannot be diminished by sorting and grading. It is therefore of utmost importance to understand biological variance, its occurrence, its origins and its behaviour throughout the entire food supply chain.

Considerable efforts have been devoted to this long time recognised problem. The main target in the well over 1 million available references (Tijsskens et al. 2000) was however put on the integration of preharvest and postharvest information, almost exclusively on dedicated problems. How can the production of this product, in these circumstances, in this production system, in this season etc. be increased with respect to quality, optimal harvesting date and mass of production, without too much interest in the processes that cause these phenomena? The more generic view on biological variance and how to avoid its burden remained largely out of focus. Even in more integrated approaches to the food supply chain the main area of integration was between preharvest and postharvest periods (Tijsskens and Vollebregt 2003).

Lately some efforts have been reported to increase the understanding of the processes involved (Baranyi 1998, Lescourret et al. 1998, Peleg 1999, Cunha 2001, Hertog 2002, Carroll 2003), and to model and describe biological variance in a deterministic way (Smout et al. 2000, 2004, Schouten 2004). Considering the repetitive use in nature of the same processes over and over again, it is not surprising that the system of problem decomposition combined with the system of process oriented modelling can provide some insight in biological variance. It even provides means of deterministic modelling of that apparently incomprehensible phenomenon. Recently, some interesting and promising studies have been reported (Schouten et. at. 2003, Schouten 2004), partly based on the theories developed in the next chapter of this work. Surprisingly, the main problem is not so much the modelling and understanding of the deterministic approach to biological variance, but rather the lack of suitable and reliable statistical methods and procedures to apply the developed theory on practical research data. To achieve a major breakthrough for converting biological variance into usefulness, this statistical technology is desperately needed. With truly interdisciplinary research between physiologists, modellers and statisticians, that is a mutual understanding of the other party's problems without forcing ones viewpoints on one another, we really can hope for a major step forward in understanding and discovering food quality matters.

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**14**

**Biological variance, burden or benefit?**

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Published in *Postharvest Biology and Technology* **27** (2003) 15-25

## Abstract

Based on theoretical considerations, a new approach to deal with the variation in properties and quality attributes inherently occurring in all products and batches is presented. It leads to a better understanding of the processes involved and enables the mathematical description and modelling of the observed phenomena. The effect of variation in harvest maturity on the composition of batches drawn from a larger population is used as an example to elucidate the effects of external conditions. These external conditions include the rate constant of the process (directly linked to temperature and controlled atmosphere (CA) conditions), the magnitude of the variation in the larger population and the size of the batches. These theoretical examples show that the standard advice of statisticians to increase the number of individuals in a batch or sample does indeed increase the reliability of data analysis based on mean values. It also shows that the so obtained estimate may well give a complete false picture of the real value. An overview is given for ways to avoid the generation of biological variance, as well as ways to decrease the effects of biological variance already present. This new approach shows how biological variance should be tackled, and even possibly turned into a benefit. A number of practical examples is provided and described briefly to underpin the validity of the approach presented. These examples relate to sources of variation (sweetening of potatoes), to the behaviour of variation during postharvest treatment (colour of apples, keeping quality of cucumbers) and to sophisticated means of dealing with biological variance without too much explicit knowledge of the mechanisms involved (dynamic control system, soil irrigation).

**Keywords:** Apple; Cucumber; Potato; Quality; Storage; Modelling

## 1. Introduction

The strategies followed in developing human skills are simple. Humans like to have a simple life, with simple rules and simple applications. Nature however, is very simple in structure, but tremendously complex in interactions. When a behaviour is encountered in nature that is not understood, its effects are avoided as much as possible. This is exactly the way we deal with the variance in our daily food. In every single item of food, grown or processed, there exists a variation in properties.

Sometimes these variations are perceived as a mere occurrence and we can tolerate them, sometimes however, the variations interfere with our intended purpose and we have to respond accordingly. Sometime these variations reveal themselves as spatial variations, sometimes as time induced variations, mostly however, the type of variation is not understood. When the observed variation is complex and difficult to understand, then the source of variation, why it occurs, is even more incomprehensible. Instead of trying to understand, biological variation and its effects on our food, the magnitude was simply kept to a minimum during production, and we learned to diminish the effects of the remaining variance as much as possible by sorting and grading.

This approach of sorting and grading worked fine for almost a century, accepting sometimes high losses, both in nutritional value (mass of product) as well as economic value (price devaluation). The changing preferences of consumers, who puts more and more emphasis on internal quality, while at the same time he persists to external appearance, forces researchers to devote more attention to the rules that govern occurrence and behaviour of biological variance. A definition of biological variance and a general description of possible sources are reported in Tijskens et al. (2000) and Tijskens and Konopacki (2003).

In this paper attention will be devoted to effects of biological variance, induced by growing conditions like, e.g. weather, season, soil and cultivation, on the storage conditions required to achieve optimal quality and keeping quality.

## 2. Biological variance, the burden

In every conceivable batch of agricultural produce, some variation in properties is present.

When this variation occurs in properties or attributes, directly or indirectly important for the acceptance or behaviour of the product, problems may arise in handling and storing the batches. Every batch will be different and will behave differently, depending on the magnitude of variation in the product and on the magnitude of the batch. To enable some description and prediction of these different batches with different behaviour, statistics has taught researchers to work with the means of sufficiently large samples. How dangerous and misleading this can be, is depicted by the following simulation experiment.

Let us consider, for the sake of the argument, the colour development in tomatoes. Tomatoes are harvested in the late green or early breaker stage (grade 4 on a range of 0 for green and 11 for red). The colour of tomatoes changes during storage (at safe temperatures) according to a logistic function (Tijskens and Evelo, 1994; Thai et al., 1990). The same logistic function has been applied to the behaviour of colour development in green vegetables (Schouten et al., 1997, 2002).

$$\text{col} = \text{col}_{\min} + \frac{\text{col}_{\max} - \text{col}_{\min}}{1 + e^{-k \cdot (t + \Delta t)}} \quad (1)$$

where col represents the colour, k the rate constant for the process of colour development and  $\Delta t$  is the maturity at harvest, relative to the breaker stage. The indices max and min refer to the maximum and minimum colour values, respectively, that can be achieved. The values of these limits depend on the colour scale used. The rate constant k is common for all individuals in a batch or even in a cultivar, while  $\Delta t$  is a stochastic variable with a different value for each individual in that particular batch.

Suppose we have a large lot of produce, offered for sale at, e.g. an auction. This lot has a variance in maturity ( $\Delta t$ ) with a standard deviation of say 5 time-units. Each time a smaller batch is drawn from the larger population, that batch will have a different composition based on the individuals involved, each with a different value for maturity.

As a result, the mean value for the colour over the individuals in a batch at any time during storage will be different for the different batches (see for example Figure 14-1). The most prominent difference between the batches is the difference in apparent rate of development,

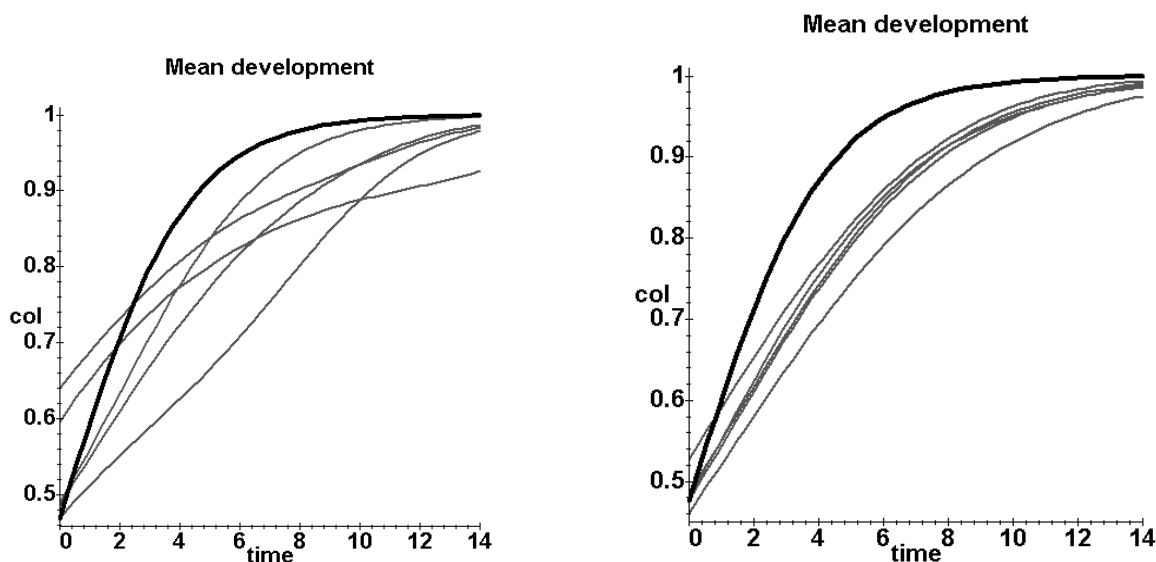


Figure 14-1 Examples of five batches of ten individuals each, taken from a population with a standard deviation in maturity of 5 time-units. The rate constant of colour development was set at 0.5/time-unit. Grey lines are simulated batches, the black line is the theoretical colour development ( $\Delta t=0$ ).

Figure 14-2 Effect of increasing the number of individuals in a batch to 100. Grey lines are simulated batches, the black line is the theoretical colour development ( $\Delta t=0$ ).

which is roughly the slope of the curve at the steepest point. Although for each individual, the colour development is simulated using the same rate constant (black line in Figure 14-1), the rate of development is highly different for the different batches. A large discrepancy exists between the successive 'repetitions' of batch formation.

Following the standard advice of statisticians, increasing the number of individuals in a batch to, e.g. 100, one indeed obtains reproducible results: the apparent rate of development of the five different batches seems almost the same (grey lines in Figure 14-2). However, we are completely put on the wrong foot. The discrepancy between observed rate of development and true rate constant used in simulation (black line in Figure 14-2) is still in the order of magnitude of 100%. Therefore, analysing results obtained from experiments and practice in this way does not provide information sufficiently reliable to warrant successful modelling of the process, let alone produce useful simulations and predictions. We have to rethink and think through the effects and behaviour of biological variance to arrive at optimal conditions in handling and storage.

The effect of the deviation in maturity at harvest ( $\Delta t$ ) on the behaviour of the mean colour value is shown in Figure 14-3 (compared to Figure 14-1). Decreasing the variation in maturity at harvest, by for example frequent harvesting or adequate sorting effectively produces the results anticipated and hoped for.

A similar line of reasoning and simulation can be applied to visualise the standard deviation in the quality attribute under study (not shown).

However, the standard deviation in maturity at harvest has a major effect on behaviour of colour development. But surprisingly, so has the value of the rate constant. An increase in rate constant (e.g. by a higher storage temperature) of only 0.5-1 has a tremendous effect on the development of the mean colour of a batch (see Figure 14-3). Lowering the temperature, or applying any other means to decrease the rate of development or physiological activity, will have a beneficiary effect on behaviour of biological variance.

It should be reminded that all these examples apply only to those properties and quality attributes that behave during their development according to a logistic function. When other mechanisms are active, different relations and a different behaviour of biological variance will be found. The line of reasoning, however, is in every case and for every mechanism the same.

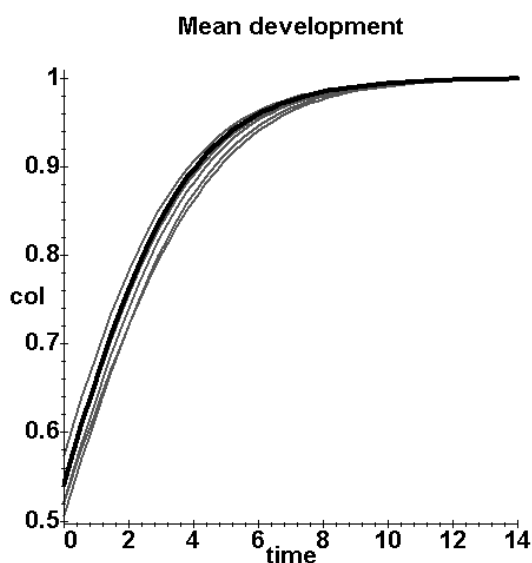


Figure 14-3 Effect of decreasing the variation in maturity to 1 time-unit, for ten individuals in a batch. Grey lines are simulated batches, the black line is the theoretical colour development ( $\Delta t=0$ ).

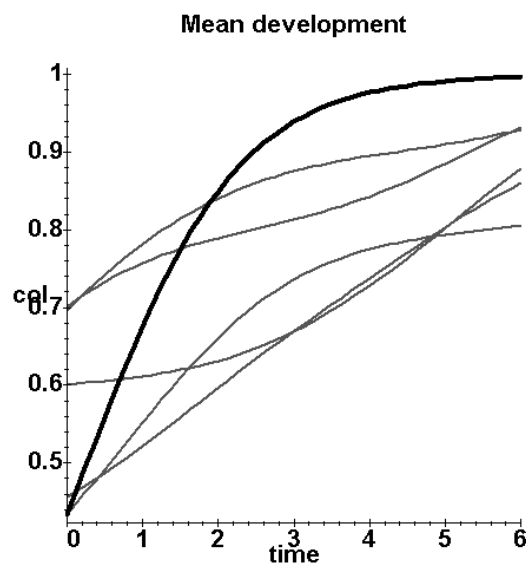


Figure 14-4 The same example as in Figure 14-1, but now with a rate constant of 1/time-unit instead of 0.5/time-unit. Grey lines are simulated batches, the black line is the theoretical colour development ( $\Delta t=0$ ).



### **3. Avoiding and decreasing biological variance**

The examples in the previous section make absolutely clear why people have tried to decrease biological variance and its effects for such a long time. The seemingly incomprehensible behaviour of product properties and quality attributes in batches of produce prevented a logical approach to the problem.

By keeping the conditions during production as constant and controlled as possible, occurrence of biological variance was diminished to a bearable level. The ultimate form of this kind of control is growing horticultural produce in greenhouses. But even in production in open field, conditions are managed to a constant level as much as possible, e.g. fertilisation, irrigation etc.

Sorting and grading further decreased the still existing variance within a population. In horticultural production, harvesting at a predefined maturity level is a form of grading applied almost intuitively. To assist growers in this respect, maturity indices and optimal harvest time algorithms have been and are being developed to indicate the point in development resembling as much as possible previous situations over successive seasons and for different growing regions (Streif 1976, 1996). Sorting and grading can, from a practical point of view, only be conducted for external quality like colour, appearance and defects. This will somewhat reduce the variation in internal quality attributes (e.g. texture, taste, flavour), but much less and much less efficient than for the external properties.

To reduce the effects of the still existing variation between batches, statistics provided research and practice with robust and reliable procedures to deal with mean values. Experimental design is tailor-made and various statistical distribution functions have been developed, along with robust procedures to use them.

All these statistical techniques, applied virtually without fundamental knowledge of the nature and behaviour of biological variance, are highly effective when comparing batches and population from similar growing conditions especially for local markets. However, comparing batches from different seasons or from completely different regions, climate and weather conditions, batch behaviour is still out of reach and incomprehensible. In view of the ongoing globalisation of the food market, and in view of the growing interest of consumers for internal quality (without neglecting appearance), these techniques are increasingly proving to be inadequate to fully comply with the needs of food supply chains in global sourcing, where the products, offered to consumers, are inherently grown under completely different conditions of climate and soils.

### **4. Biological variance, the benefit**

That biological variance is a major issue for the improvement of sensory and production quality of our food has long been recognised. A vast effort has been devoted in the areas of growing and sorting. A simple search through the electronic libraries, which only cover the last 40 years, reveals more than 1 million reports (see for a few examples Tijskens et al., 2000). This stresses the importance of the problem that has been recognised for many years. The result of most of these studies is more an enumeration of observed phenomena, rather than an interpretation in terms of processes. Although to our knowledge, a fundamental approach to evaluate these data has never before been conducted, in the end only a fundamental approach will provide the benefits of biological variance. The scientific world and modern technology is full of examples of successful applications of processes that were previously unknown and not understood. As soon as part of the biological variance is understood, we find new applications for it, and it ceases to be regarded as biological variance. The variation that exists among cultivars of one species is successfully applied for increasing the range in taste and appearance of our fresh food.

The way in which the understanding of the dynamics of biological variance will provide benefits for agriculture, trade and consumer is presently not clear. It is, however, evident that in research as well as in daily practice, we have to avoid working with mean behaviour altogether (see previous examples) to advance knowledge and understanding of product behaviour and quality optimisation. Although based entirely on the properties of individuals,

biological variance is a property of a population. Attention should therefore be devoted to the behaviour of individuals rather than to the entire population as a whole. With information on that level, the fundamental processes of development and decay can be unravelled and studied in more detail. Knowledge about these generic and fundamental processes will inevitably lead to practical applications.

In the next paragraphs some examples from practical research are briefly explained and used to elucidate the line of reasoning and the consequences for research and practice.

#### 4.1. Colour of apples in storage

The development during storage at three different temperatures of the colour of apples (cv. 'Granny Smith'), harvested at two stages of maturity at three different orchards in Slovenia, could be described in a generic fashion with a logistic function, similar to Eq. (1). All data could be pooled and analysed together, provided the initial condition of each individual apple was taken into account. The obtained percentage variance accounted for ( $R^2_{adj}$ ) was more than 95% (Tijskens et al., 2000, 2003).

In these analyses, the kinetic parameters were again estimated in common for all batches and individuals, the initial conditions ( $col_{min}$  in Eq. (1) and  $\Delta col_0$  in Eq. (2)) were estimated for each individual apple. Assuming a normal Gaussian distribution on the harvest maturity  $\Delta t$  a skewed distribution on the quality attribute colour can be deduced. In Figure 14-5 an example is shown for the statistical density that an apple is in certain colour stage at different times of storage. When the apples are very young (left most curve) the majority of the apples are within 20% of the total colour range. On a visual scale of 0 (green) to 1 (yellow), this means only two-tenths of a unit. The naked human eye can hardly see any difference between these apples. However, the few apples in the batch that are less green (colour difference more than 0.2) determine for the major part the acceptability and the keeping quality of the whole batch (Schouten et al., 1997). During storage, the distribution becomes less skewed (more Gaussian) but flatter and wider, with a much higher standard deviation (Figure 14-5 middle curves). The distribution again becomes skewed at the end of storage (right most curves) when all apples are more or less yellow. At this time, the batch of apples has no economic value.

To predict colour development and associated keeping quality (Schouten et al., 1997; Tijskens and Polderdijk, 1996), the most important factor proved to be the difference between the actual colour and the most green colour possible ( $\Delta col = col - col_{min}$ ). Transforming all measured data with this relation, the colour of apples was indeed found to exhibit a skewed distribution (Figure 14-6).

For the initial colour (time=0) on a normalised scale with  $col_{max}=1$  and  $col_{min}=0$ , Eq. (1) reduces to Eq. (2).

$$\Delta col_0 = \frac{col_{max} - col_{min}}{1 + e^{-k \cdot \Delta t}}$$

$$\Delta t = -\frac{\ln\left(\frac{1}{\Delta col_0} - 1\right)}{k} \quad \text{eq 2}$$

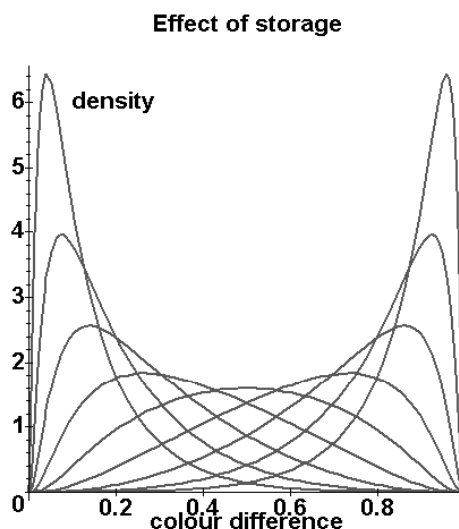


Figure 14-5 Theoretical density of colour differences ( $\Delta col_0 = col_0 - col_{min}$ ) and their development in time, rate of colour development 0.5/time-unit, standard deviation in harvest maturity 2 time-units.

The change during storage in the shape of the distribution of colour difference in apples is strikingly similar to the shape of the theoretical distribution.

The processes occurring in the apple, changing their colour during storage and maturation, not only determine how and how fast the distribution will change in shape and maximum density, but also the generic shape of the distribution. It is well known in statistics and population dynamics that a logistic function has to be coupled with a binomial distribution, which resembles very much the theoretical behaviour. Equally so, the Poisson distribution is intrinsically connected to exponential behaviour. When we extend this view, we can say that each mechanism has its specific distribution function. Each specific combination of processes working in perishable products will determine not only the kinetics of the properties involved, but also the type, shape and dynamic behaviour of the distribution of the properties of that product. Each process occurring in perishable product generates its own particular distribution with its own shape and its own dynamic behaviour. Based on theoretical considerations, a procedure has been developed to deduce these distributions each linked to a specific process (or more precisely its model formulation) and to describe their general type, shape and dynamic behaviour. However, the results are not yet fully understood. Research continues.

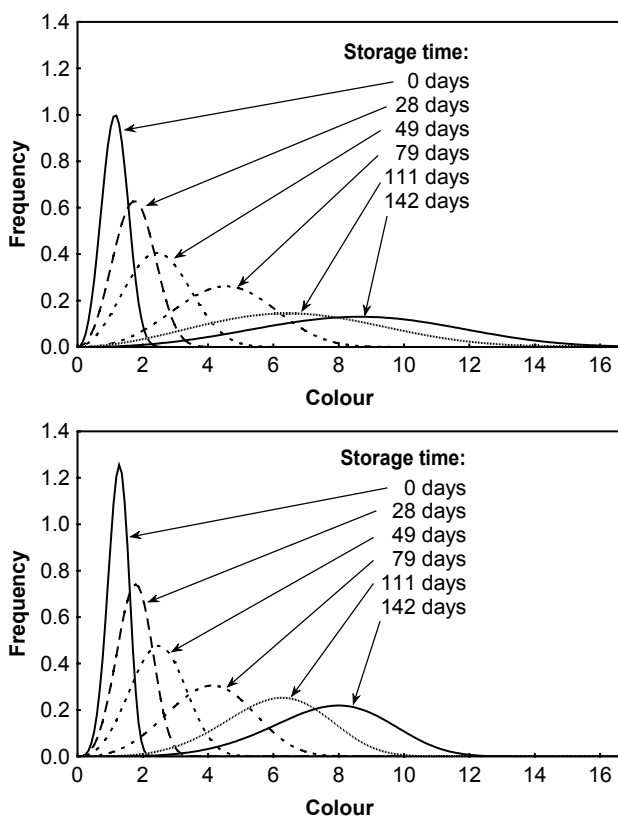


Figure 14-6 Distribution in colour differences ( $\Delta col_0 = col_0 - col_{min}$ ) of apples from an orchard at Krško (Slovenia) at early (top) and mature (bottom) stage of maturity and stored up to 142 days at 4-8°C. Approximated with a Weibull distribution.

#### 4.2. Sweetening of potatoes

The content of sugars was measured in batches of potatoes, harvested over many seasons, and for many cultivars (Hertog et al., 1997a,b). The same generic and physiological model was applied in a non-linear regression analysis of the data obtained for all seasons and cultivars. It was found that for each cultivar separately, the same value for the kinetic parameters (rate constants and activation energies) could be applied over the successive seasons. The only batch parameter (all initial levels of ingredients) that was clearly different for batches from the successive seasons was a lumped (unspecified and therefore not measurable) enzyme system. These findings could be validated on a completely separate

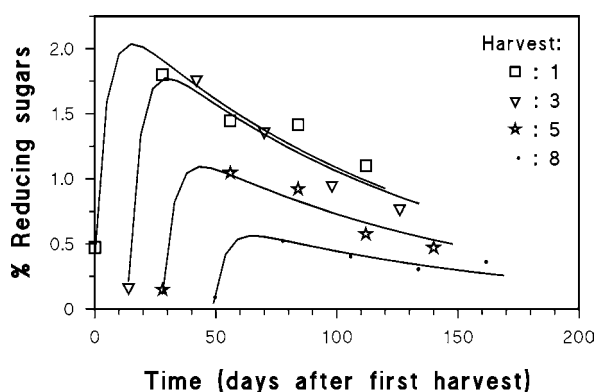


Figure 14-7 Sugar accumulation in potatoes at successive stages of harvest maturity (Hertog et al., 1997a). Symbols are measured data, lines are data, simulated with the same model and the same parameter values except for the lumped enzyme system.

set of data. Therefore, the complete effect of harvest maturity (per cultivar) could be traced back to the level of that one lumped enzyme system (see Figure 14-7). The level of the lumped enzyme could be determined by accelerated testing early after harvest. This information can be used to determine acceptable storage conditions. Research on the nature of that lumped enzyme system (van Hoof, Unpublished results) is continuing.

### 4.3. Keeping quality of cucumbers

The conditions applied during growing of agricultural produce have a marked effect on postharvest behaviour of those products. A study on batch variability in colour and colour development of cucumbers was conducted to check the type and magnitude of these anticipated effects (Schouten et al., 1997; Schouten and van Kooten, 1998). The differences in colour development expressed in a way, similar to the apple example ( $col - col_{min}$ ), were again too small to be visible by the naked eye. Marked differences between batches from four different growing conditions were found in both the shape and skewness of the colour distribution curves (see Figure 14-8). As colour development could be described by the same model used for the apples, the same type of behaviour could be expected. What was sought in this study was a relation between the skewness of the colour distribution, the limit of acceptance of a batch and the expected keeping quality or shelf life of that batch. The expected keeping quality of cucumber batches could be linked to the skewness of the colour distribution, indiscernible to the naked human eye. The more skewed the colour distribution at the moment of harvest, the longer it will take for the first individuals to become unacceptable, and the batch will have a longer keeping quality or shelf-life. These results

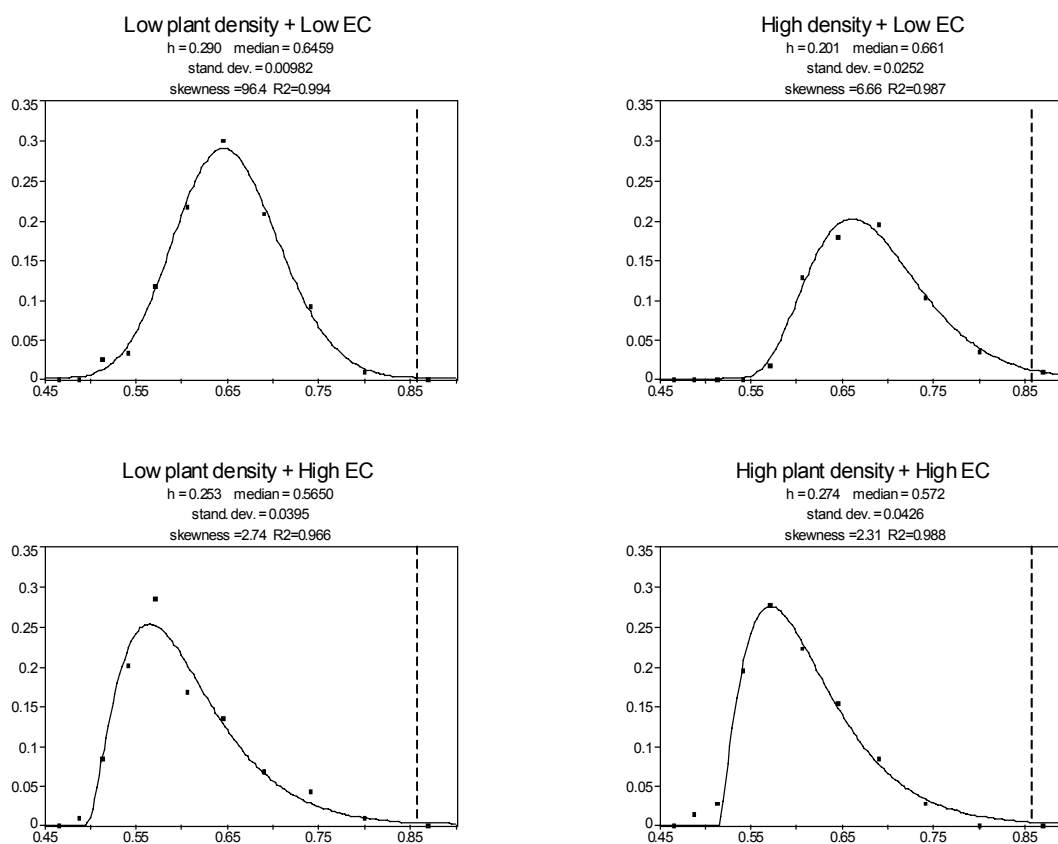


Figure 14-8 Colour distributions in cucumbers from four different treatments. Values on the X-axis represent the colour (expressed as colour card value) and values on the Y-axis represent the probability. (The X-axis runs from left green to right less green, more yellow.) The colour limit (dashed line) is indicated. The distributions were fitted with an empirical gamma function (Schouten and van Kooten, 1998).

were recently validated and extended by using an improved colour measuring system, based on computer imaging (Schouten et al., 2002).

#### **4.4. Soil moisture deficit and irrigation**

From the beginning of farming one of the aims was to understand observed phenomena and to find ways to increase yield. Very soon, the importance of water in food production was recognised. Although the relation between weather and irrigation is obvious to us, in fact it took several thousands of years before humans tried to predict and quantify the need for irrigation based on measurements of environmental parameters. One of the earliest investigations on evapotranspiration was conducted by Penman (1948). A wide range of research projects originated from Penman's idea and his equation, which is based on sound and accepted physical principles. The most important follow-up work was reported by Monteith (1965) who extended Penman's formula. The results of his study eventually led to the problem posed by the consequences of biological variance.

The Penman-Monteith formula describes the transpiration rate not only in terms of meteorological parameters but also in terms of physiological resistance to water movement within the plant and between plant and ambient air. This formula explained some of the reasons, observed for long time, of difference between temperatures of plant leaves and ambient air. It has greatly improved the understanding of the relation between these temperature difference and soil moisture deficit.

Further research converted this knowledge into practical applications. Some irrigation systems (usually of self-propelled types like centre pivot or lateral) have been equipped with infrared thermometers to detect (locally different) water stress and deliver a (locally different) appropriate amount of water accordingly. This system is usually associated with precision agriculture but nowadays its aim is to enhance product quality or reduce variation rather than to increase yield. Research in this field is continuing.

#### **4.5. Dynamic control system**

Recent developments in dealing with difference between batches of apples and pears have lead to the development of a dynamic control system reported by Veltman et al. (2003), van Schaik & Verschoor (2003), Oosterhaven (2003) and Nicolai et al. (2003). In this system, the controlled atmosphere conditions are adapted to the actual response of the product in terms of fermentation and measurable fermentation products (due to hypoxia). The actual state of the product is monitored, and the (mean) response of the product is used to control the conditions, virtually without knowledge or understanding of the dynamics of the actual processes that generated that response.

Although this system works with responses generated by a whole batch during storage, again the most deviant individuals, that are located in the skewed part of the distribution of the property (in this case fermentation), determine for the major part the overall response. Effectively this signifies that the most sensitive individuals determine the conditions of the whole batch. As a result, the adaptation of the controlled atmosphere (CA) conditions to the properties of the stored batch results in improved quality retention, while the number of individuals that become unacceptable is very low.

### **5. Consequences for storage conditions**

Optimisation can be achieved without explicit knowledge of processes, mechanisms and rules involved in the storage of agricultural produce (see for example Section 4.5). Understanding of the sources and effects of variation present in all batches of produce directs research efforts to more efficient tools and means for developing practical solutions. When the sources, the reasons of existing and the behaviour of the variance can be modelled and predicted, optimisation of quality, in terms of preserving eating quality while avoiding losses by storage disorders, will no longer be like searching for a needle in a haystack. Attention can then be devoted directly to the underlying processes.

Of course, this more fundamental way will take quite some time to develop fully. We have just begun to understand how and why biological variance is so important. The classical approach took more than 60 years of continuous attention to achieve the impressive results obtained in the last couple of decades.

Some practical hints can already be deduced from present knowledge.

- Application of standard statistics, analysing mean data of batches, may be dangerous and misleading. Discrepancies in composition of batches may well be the reason for the sometimes large differences in parameter values, reported in the literature.
- Lowering the rate of physiological processes during storage will also lower the increase in variance in a batch. It is not important how the physiological rate is lowered. Lowering storage temperature as well as lowering the respiration rate by a lower oxygen concentration or a higher carbon dioxide concentration are both equally effective. In fact, it is most likely that CA and modified atmosphere (MA) storage were found to be so effective by the sheer coincidence that lowering the rate of physiological processes also stabilises existing biological variance.
- Adequate and sensitive techniques for measuring quality related properties will provide the necessary means to detect and evaluate the skewness of distribution in batches, which can be directly connected to the acceptability and keeping quality of batches of agricultural produce.
- The proposed approach to biological variance intrinsically offers the possibility and the means to link postharvest behaviour to preharvest growing conditions. More research and especially more fundamental models are needed to achieve prediction of postharvest behaviour independent of season and region.

## Acknowledgements

The many discussions on biological variance in all his aspects with colleagues M. Hertog, E. Biekman, R. Schouten, C. van Dijk and G. Jongbloed are gratefully acknowledged. The authors thank the editors in chief of Postharvest Biology and Technology for the kind invitation for this paper.

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## **Part 7**

### **QUALITY IN RESEARCH**

## Introduction

The advantage of short stories and fairy tales on certain scientific subjects is that one can and may express ones views on a certain subject more freely, without the straitjacket of the structure of scientific publications. Short stories and fairytales also provide the means of taking some distance on the subject at hand. By writing fairytales, one is encouraged to generalise the subject to the core of the matter. Difficult subjects can be discussed and communicated to readers and audience otherwise afraid of paying attention.

In the next chapters some viewpoints on modelling and generalisations were expressed, that have been read as part of the opening of conferences. They not only reflect the power of modelling but also the weakness of understanding and modelling in the realm of economics, commerce, psychology and art.

**15**

**The model of the golden coins.**

**L.M.M. Tijskens, M. Tijskens-Leensen, M. Simčič**

Published in Proceedings *First international Symposium Model-IT*, December 1998, Wageningen, NL. *Acta Horticulturae* (1998). **476** p. 11-12

Once upon a time an old wise farmer lived in a small village, growing his crops and selling them at the local market to the people in the neighbourhood. He had two sons and a daughter who helped him with the growing and the selling of the products of his land.

When the eldest son reached the age to leave the farm where he spent all of his youth, and to start a career of his own, he went to his father and said, 'Father, I want to take off into the wide world and search for me my own place and family.' And the old father, wise as he was, agreed with the young man that it was his time to start something new. 'What do you want to do with your life?', he asked his son. And the son said, 'I have seen you talking to the people in the village and advising everyone who sought your wisdom. And I have seen you working at the marketplace, selling all the crops you grow on your land and I learned from you how to do that. So, I want to work with people and go into business, sell anything the people want to have, and become rich and wealthy'. As all fathers are, the wise man was mighty proud of his son's words. And he said to his son: 'You made a good choice, my son, you really have the way with people'. To help his son to make his dreams come true, he gave his son a bag of coins. But one coin, a golden one, he gave his son directly into his hands and said: 'Take this golden coin with you and cherish it for it is a model of wealth and richness'. And the son took off to Big City, worked very hard and became a wealthy and respected sales manager. He did not need to go wandering around the world, as all the precious goods of the world came to him to be sold. Often he sat with the golden coin of his father in his hands. And he saw his world of wealth in the hard coin

When the second son reached the age to leave the farm where he spent all of his youth, and to start a career of his own, he went to his father and said, 'Father, I want to take off into the wide world and search for me my own place and family.' And the old father, wise as he was, agreed with the young man that it was his time to start something new. 'What do you want to do with your life?', he asked his son. And the son said, 'I have seen you and the other people at the marketplace, flamboyant in the sun and melancholic in the rain. I want to catch that feeling and paint people and landscapes in the marvellous ever-changing light.' As all fathers are, the wise man was mighty proud of his sons words, and a little bit anxious at the same time for his son's future. And he said to his son: 'You made a good choice, my son, you really have the way with expressing the marvels of the world'. To help his son to make his dreams come true and to help him through the near future, he gave his son a bag of coins. But one coin, a golden one, he gave his son directly into his hands and said: 'Take this golden coin with you and cherish it for its design is a model of beauty in a simple form'. And the son took off to Flower Town, worked very hard and searched the changing light in Saint Francis Land, and the beauty of the Vanishing Lake and the Lovely River. He became a world famous and respected painter of light and portraits. Often he sat with the golden coin of his father in his hands. And he saw his world in the simple design of the bright coin.

When the youngest one, his beloved daughter, also reached the age to leave the farm where she spent all of her youth, and to start a career of her own, she went to her father and said, 'Father, I want to take off into the wide world and search for me my own place and family.' And the old father, wise as he was, agreed with the young woman that it was her time to start something new. 'What do you want to do with your life?', he asked his daughter. And the daughter said, 'I have seen you growing the products of your land. And I have seen the wondering in your eyes when you looked at them. I want to know and understand the wonders of nature'. As all fathers are, the wise man was mighty proud of his daughter's words. But now he was quite anxious at the same time for his daughter's future for he did not know what had to become of her. And he said to his daughter: 'You made a good choice, my daughter, you really have the way of knowing the living creatures on this earth.' And to help his daughter to make her dreams also come true and to help her through the future, he gave his daughter a bag of coins. But one coin, a golden one, he gave his daughter directly into her hands and said: 'I cannot give you a model for what you search. You have to make your own way into this land of study and understanding'. And the daughter took off to Knowledge

Centre in the Low lands at the Sea, and studied very hard for a long time. And after many years she understood nature well enough to make her own models of living and dying. And she became famous for her models and for her understanding of nature. People all over the world, sought her advice in understanding, and invited her to visit them and to teach them how to understand nature. Often she sat with the golden coin of her father in her hands. And she saw the real world in the mirror of the shining coin.



**16**

**The Princess and the doll**

**Pol Tijskens**

Published in Proceedings *Second international Symposium Model-IT*, December 2001,  
Palmerston North, NZ. *Acta Horticulturae* **566**, 19-20

Once upon a time, a magician with dolls made a very fine doll, with an alabaster face and with arms and legs that could move. He put his skills in the making of the doll. When he saw the results of his effort, he was proud of himself and he thought: "This is such a fine doll, with nice flexible arms and legs. I will go and give it as a present to our princess, high up in the castle."

He packed the doll in a fine wooden box, packed his clothes, packed something to eat and started on his journey to the castle. When he arrived at the castle, he went to the princess, and said: "My beloved princess, I am the magician with dolls, and I made specially for you a nice doll with an alabaster face and arms and legs that can move". The princess took the doll, and was very pleased with it. For a couple of days. But after a couple of days she thought: "This doll is not really very amusing. She only has an alabaster face and arms and legs that can move. I want another doll that can do more. Then I will be pleased".

And she called upon the doll magician and said: "This is a nice doll with an alabaster face and arms and legs that can move. But there is something missing. I am no longer pleased with the doll".

The magician was a little bit sad that the princess did no longer like his masterpiece doll. He took the doll back to his magic workshop, and stared at it for a couple of days. And he said to himself: "Let me give her face a fair complexion, like the colour of our princess's face, and make the doll so she can walk and move her arms herself". And so he did in his magic workshop. He put in the making of the doll more of his skills. When he saw the results of his effort, he was very proud of himself and he thought: "This is such a fine doll that can walk and move her arms. I will go and give it as a present to our princess, high up in the castle."

He packed the doll in a fine wooden box, packed his clothes, packed something to eat and started again on his journey to the castle. When he arrived at the castle, he went to the princess, and said: "My beloved princess, I made specially for you a nice doll with a fair complexion. She can walk and move her arms herself". The princess took the doll, and was very pleased with it. For a couple of days. But after a couple of days she thought: "This doll is not really very amusing. She has only a fair complexion and she can move her arms and legs herself. I want another doll that can do more. Then I will be pleased".

And she called upon the doll magician and said: "This is a nice doll with a fair complexion that can move her arms and legs by herself. But there is something missing. I am no longer pleased with the doll".

The magician was quite sad that the princess did no longer like his masterpiece doll. He took the doll back to his magic workshop, and stared at it for a couple of days. And he said to himself: "Let me give her a voice of herself, so she can speak and think for herself". And so he did in his magic workshop. He put in the making of the doll all of skills. When he saw the results of his effort, he was mighty proud of himself and he thought: "This is such a fine doll that can speak and think for herself. I will go and give it as a present to our princess, high up in the castle."

He packed the doll in a fine wooden box, packed his clothes, packed something to eat and started once more on his journey to the castle. When he arrived at the castle, he went to the princess, and said: "My beloved princess, I made specially for you a nice doll that can speak and think for herself. Now I am sure you will be pleased with my doll". And the princess took the doll, and was indeed very pleased with it. For a couple of days. But after a couple of days the doll said to the princess: "You are just a princess, who cannot think for herself, and who is a nuisance for everybody around you. I am no longer please with you. I want to go back to my magician".

And she called upon the doll magician, and went back with him to the magic doll shop and was happy with her magician for the rest of her life.



**Legend of symbols<sup>1</sup>**

<sup>1</sup> Only for those who do not understand

Symbol	Meaning
Doll	Model
Magician	Model builder
Magic workshop	Computer
Princess	Manager
Castle	Commercial company
Face	Model interface
Fair complexion	User friendly model interface
Arms & legs	Predictions
Walk	Stand alone model
Think	Generic model prediction



## **Part 8**

### **CONCLUDING REMARKS**

Quality is diverse, not only among and between people and products, but also in research subject. The selection of chapters in this work clearly supports that fact. A broad range of food products, quality attributes and product properties has passed by. On a first glance it is just a loose conglomerate of past research in a man's career. However, combining that loosely connected information with a process oriented modelling effort, a large number of similarities does emerge. Apparently, the same processes, and a very limited number for that fact, appear in nature over and over again in an almost infinite number of situational combinations.

The decomposition of quality into on the one hand an assigned quality, solely defined by the state of the product, and the other hand an acceptability for the consumer / user, determined by the assigned quality and the social and economic circumstance of that user (Part 2) does allow to model and understand the quality behaviour of any perishable product. The development of a more fundamental type of modelling (Part 1), which is also based on problem decomposition, greatly enhances the progress made in understanding quality and quality behaviour in food products. The combination of both viewpoints in conjunction with a meticulous application of the rules of nature and disciplines, allows a clearer view of the issues that are really at stake in our ongoing strive for more knowledge and more quality. It can help research to target at the real problems, and thereby conduct research with more efficiency and with more efficacy (chapter 2). In a time of limited budgets for research, this seems rather important. Food research can simply no longer afford to spend its limited financial resource on empirical research for years on end. Empirical research, which is searching for solutions without the guidance of some form of theory, seems to deliver rapidly applicable solutions (short term solutions) while research along a more fundamental line of thinking seems to deliver solutions only in the far future (long term solutions). When counting the combined efforts over the years, however, fundamental oriented research based on the guidance of theory and modelling, using all knowledge available, empirical and theoretical alike, delivers in the long run solutions and applications in a far more efficient manner.

Up to now the major contribution that can be provided by modelling is not so much the applications in practice, but exactly that guidance of future research. In that sense, modelling is really discovering the future of food quality matters.

With the increasing importance of quality issues for the modern consumer, and the increasing importance of world wide buying of food and transporting it around the globe, good and fundamental knowledge and reliable and reusable models on product behaviour and product quality will become, or already are, an absolute necessity. In this compilation of research over several years based on knowledge and data already available, it is made clear not only that fundamental and generic modelling can be done but also how it can be achieved. Imagine what this system could deliver when experiments are conducted according to these viewpoints. The modelling and analysis of data obtained in that way would result in truly intelligent and interdisciplinary interpretations and applications for discovering the future in food quality matters.

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## Curriculum Vitae

Pol Tijskens was born in Mechelen (Belgium) in 1942. As the youngest in a family of 11 children, all interested in nature, science and logic, he learned naturally to acquire all kinds of information, useful as well as useless.

He finished secondary school (Greek and Latin) at the Sint Rombouts College in Mechelen in 1960. Further scientific education was acquired at the Katholieke Universiteit Leuven (B) where he graduated as Licentiate in Chemistry, direction Organic chemistry in July 1964.

He started his professional career at the research institute NITHO (Nederlands Instituut voor Toegepast Huishoudkundig Onderzoek) in Wageningen, the Netherlands in August 1965, working on textiles and washing agents.

In October 1973 he switched to fruit and vegetables at the Sprenger Institute (Instituut voor Bewaring en Verwerking van Tuinbouwproducten), and studied there for 17 years the mechanical properties of fruits and vegetables, both fresh and processed. At the same time he was active as food process engineer in the heat sterilisation and blanching of fruits and vegetables. In that period a vast latent knowledge was built up in the area of behaviour and quality of horticultural food. Also in that period, an interest was developed in computers, automatic data analysis and modelling.

In 1988, the Sprenger Institute was abolished, and he took up the position of mathematical modeller, specialised on quality behaviour of fruit and vegetables in the newly formed Institute of Agro Technological Research (ATO, now Agrotechnology & Food Innovations BV,). In building all those models, all that latent knowledge, his mathematical and chemical skills came together with his interest in nature, and the fundamental thinking he acquired over the years, which resulted in about 40 published papers, 10 chapters or books, and 50 lectures and posters.

## Abstract

Quality of agricultural products becomes increasingly important to consumers, and hence to producers and retailers. Quality, however, is ill defined and perceived and evaluated differently by different individuals, or by groups of individuals. Based on problem decomposition, a working theory on quality of agricultural produce was developed, taking the variability in liking and evaluation of quality among humans into account. The realm of quality is decomposed into one part predominantly connected to intrinsic product properties, one part predominantly linked to consumer behaviour and appreciation, and one part predominantly linked to economic and market effects. The first item is covered by this study, the second part is the area of sensory, consumer science and psychology of man, and the last part is covered by market research and economics.

Equally important to satisfy consumers with good quality produce while maintaining a competitive position in the market is modelling of quality behaviour. Proper and reliable simulation and prediction of food quality allows the optimisation of the production and distribution process. Based again on problem decomposition, a process-oriented modelling approach was developed, that uses all available information, not only that contained in experimental results, but also the knowledge of experts and theory.

Based on both these theories and techniques and to prove their usefulness and added value, a number of models are developed and described covering the processes, intrinsic product properties and attributes that are important in postharvest technology (enzyme activity and denaturation, respiration and fermentation, chilling injury and free radical scavenging, firmness decay) and in the processing technology (effect of blanching on firmness and colour).

For the distribution and logistics and to include some aspects of the psychology of food acceptance by consumers, the model of keeping quality is described which was developed by including some theoretical considerations into the old, well-established but empirical system of shelf-life.

All these processes, models and system are prone to unknown and not understood effects of biological variance, both in product properties and behaviour, as in consumer liking and acceptance. A systematic approach to biological variance is in full development, making it possible to include stochastic effects in deterministic models and in statistical analysis.

## Samenvatting

Kwaliteit van ons voedsel wordt steeds belangrijker voor de moderne consument. Daarom besteden producenten, groothandel en detailhandel steeds meer aandacht aan kwaliteit. Kwaliteit is echter zeer moeilijk en dus slecht gedefinieerd. Het wordt door iedereen anders waargenomen en beoordeeld, niet alleen door individuele personen, maar ook door groepen personen.

Door probleem decompositie toe te passen op kwaliteit werd een werkbare theorie opgebouwd, die rekening houdt met de individuele en groepsverschillen in kwaliteitsperceptie en -evaluatie. Het gehele gebied dat kwaliteit bestrijkt, kan worden opgedeeld in één deel dat hoofdzakelijk verband houdt met de intrinsieke eigenschappen en de daaruit afgeleide kwaliteitsattributen, één deel dat hoofdzakelijk verband houdt met consumenten gedrag en waardering en één deel dat hoofdzakelijk verband houdt met economische en markt invloeden. Het eerste deel wordt in deze studie behandeld, het tweede deel bestrijkt het gebied van sensorisch onderzoek, consumenten onderzoek en de psychologische effecten in de mens, het derde deel beslaat het marktonderzoek en de economie.

Om in staat te zijn ook in de toekomst aan de wensen van de consument tegemoet te komen en zich gelijktijdig van een marktaandeel te verzekeren, is een betrouwbare voorspelling van het te verwachten product gedrag zeer wenselijk, zo niet noodzakelijk. Gedegen en betrouwbare modellen maken het pas mogelijk de voedselketen zowel in productie als in distributie te optimaliseren. Hiertoe is een systeem ontwikkeld, gebaseerd op probleem decompositie en procesgeoriënteerd modelleren, dat alle beschikbare informatie gebruikt, niet alleen gegevens uit experimenteel onderzoek, maar ook de specifieke kennis van experts en van de bestaande theorieën.

Gebaseerd op deze twee theorieën en technieken worden een aantal modellen ontwikkeld en gepresenteerd. Deze modellen beslaan het gehele gebied van intrinsieke product eigenschappen en de bijbehorende kwaliteitsattributen welke van zo'n groot belang zijn in naoogstfysiologie als enzym activiteit, enzym denaturatie, ademhaling en fermentatie, koude schade en het neutraliseren van vrije radicalen en stevigheidsverlies, en voor processing technologie de effecten van hittebehandelingen op stevigheid en kleur.

Wanneer we de kwaliteit in distributie en logistiek behandelen, komen we dicht bij de consument, en moeten we dus een deel van het psychologische invloeden meenemen in de modellen. Het model van de houdbaarheid werd ontwikkeld uit het welbekende maar empirische systeem van shelf-life door er enige theoretische overwegingen aan toe te voegen.

Al deze processen, modellen en systemen zijn onderhevig aan onbekende en meestendeels onbegrepen effecten van biologische variatie, zowel in productgedrag als in consumentengedrag. Een systematische aanpak van dit probleem van biologische variatie is in volle ontwikkeling. Deze aanpak maakt het mogelijk om stochastische effecten mee te nemen in deterministische modellen en in de statistisch analyses.

## Dankwoord

Terugkijkend op bijna veertig jaar actief onderzoek vind ik het zelf nog het meest verbazingwekkend dat dit uiteindelijk tot dit proefschrift heeft geleid. Jarenlang was een proefschrift geen moment in gedachten. Ja, het kon wel, maar het hoefde toch niet! Tot een paar jaar geleden, Erna eens opmerkte dat ze daar toch wel trots op zou zijn. En dat zette me echt aan het denken. Een promotie is niet alleen voor jezelf, maar ook voor de mensen om je heen! Erna was en is wel vaker de directe aanleiding geweest om mijn praktische kennis bij te spijkeren, en was altijd de eerste praatpaal en vraagbaak. Erna, bedankt voor de vele fijne jaren en de onaflatende stilzwijgende steun.

Uitgerekend in Nieuw Zeeland kwam Olaf er achter dat wij daarover gesproken hadden. En met zijn welbekend enthousiasme en kameraadschap, bleef hij aandringen tot het hoge woord eruit was. Olaf, bedankt voor alle steun, bewondering maar vooral voor al het onafgebroken vertrouwen dat je in de loop van vele jaren getoond hebt.

Uiteindelijk heeft dit proefschrift dus bijna 40 jaar gevergd. Het aantal personen dat in meer of mindere mate hieraan hebben bijgedragen is dus ook te groot om ze allemaal op te noemen. Een aantal wil ik hier toch noemen, omdat hun inbreng niet weg te denken is.

In de eerste plaats de leiding van het instituut ATO, nu Agrotechnology and Food Innovations, en de Business Units waar ik gewerkt heb. Ze hebben me al die jaren toegestaan en gestimuleerd fundamentele modellen te maken, en mij niets aan te trekken van de traditionele muurtjes die nog overal opgetrokken worden. Koos, voor mij ben jij de persoon waarin al die leidinggevers tezamen komen. Bedankt. Maar één persoon in die grote rij wil ik toch met name noemen, omdat hij mij, in de eerste jaren van het ATO, de ruimte heeft gegeven het systeem te ontwikkelen waarop dit proefschrift geheel is gebouwd: Peter Reinders, bedankt.

In de tweede plaats, wil ik alle (mede-) auteurs bedanken voor hun steun, medewerking en vertrouwen. To all my (co-) authors of papers and lectures, thank you for the trust in me and for all your willingness and enthusiasm. Some of you I never met, many of you are now good colleagues, and some of you became very good friends for life.

In de derde plaats, alle collega's die altijd een welwillend oor hadden om naar mijn onorthodoxe vragen en problemen te luisteren, en altijd bereid waren mee te denken en een goede wetenschappelijke discussie te voeren. Maarten, Rob, Elvis, Cees, Olaf, Wouter, Mark, bedankt.

In the last place, but by no means the least, I want to express my special gratitude to the two guys that always believed in me, even when they not always understood what I was talking about. Marjan najlepša hvala, Paweł dziękuję bardzo. Your friendship, discussions, questions and belief was absolutely necessary to achieve this final step. Thank you so much. Ook mijn kinderen wil ik bedanken, dat ze er waren, dat ze wilden luisteren naar al die verhalen over modellen, artikelen en boekjes. Bart en Marieke, Tonny en Linette, bedankt. Tenslotte wil ik nog vermelden dat kleinkinderen een voortdurende bron van inspiratie zijn, vooral voor het ontwerpen van acroniemen voor modellen. Jessica, Bram en Melissa, ik hoop dat jullie later nog net zo trots kunnen zijn op Vava, als ik nu op jullie ben.

Wageningen, december 2003



## Colophon

The pictures used for front and back cover do need some explanation.

Both pictures are dear memories of trips to postharvest conferences, kindly provided by the Institute Agrotechnological Research Organisation (now Agrotechnology & Food Innovations) where I worked so long on modelling. So, both pictures are meant as a tribute to the Institute.

The front picture is a sunset approaching Cordoba from Granada in the South of Spain. When I started on this thesis, some light was still on my career.

The back picture is a silver-light full moon over Leasure Island in the Bay of Plenty, taken at the last evening of a two month trip to New Zealand. When I finished this thesis, I reached the end of my career, although not in complete darkness.

## Colofon

De foto's op de voor en achter kant van dit boekje hebben enige uitleg nodig.

Beide foto's zijn dierbare herinneringen aan uitstapjes naar naogst conferenties, mogelijk gemaakt door het Instituut ATO (nu A&F), waar ik zo lang mocht modelleren. Het gebruik van deze foto's is daar dan ook een dankbetuiging voor.

De foto op de voorkant is een zonsondergang boven Cordoba, naderend vanuit Granada in Zuid Spanje. Toen ik aan dit proefschrift begon was er nog enig licht in mijn carrière.

De foto op de achterkant is het zilverlicht van een volle maan boven Leisure Island in de Bay of Plenty, genomen op de laatste avond van een trip van twee maanden naar Nieuw Zeeland. Nu dit proefschrift klaar is, heb ik het einde van mijn carrière bereikt, alhoewel niet in volledige duisternis.