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Third Annual Progress Report

Mealiness in fruits

Consumer perception and means for detection

Report Period 01.01.1997 – 30.04.1999

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Report Period	01.01.1997 – 30.04.1999

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INTRODUCTION

Texture, with emphasis on "mealiness" is a very important quality attribute for fresh fruits. Especially the rapid and non-destructive assessment of this quality attribute is of main importance. For this reason two fruits were chosen, tomatoes and apples, in order to research and establish the relations between rapid, non destructive analysis, with emphasis on Near Infra Red (NIR) spectroscopy, and this quality attribute. For these two fruits different strategies were chosen.

Apples
Apples of several varieties, including Cox's Orange Pippin, Jonagold, Starking and Elstar were, after harvest, stored at their optimal conditions. This storage regime was broken, followed by a storage regime known, to evoke mealiness. Apples were sampled during this process of mealiness development, at a range in their degree of mealiness. These apples, varying in their degree of mealiness, were further analysed. This analysis comprised:

Destructive analysis:
- sensory analysis, using a trained analytical sensory panel. In some cases an expert panel was used,
- analysis of the major, non-volatile taste components; sugars and organic acids,
- analysis of the major, volatile taste components.

Non-destructive analysis on individual apples:
- NIR spectroscopy of the intact apple samples.

On basis of this information matrix, correlations were established and calibration curves designed relating:
- NIR spectra and sensory texture attributes,
- NIR spectra and sensory taste attributes,
- NIR spectra and non-volatile taste components,
- Volatile and non-volatile taste components and sensory texture attributes,
- Volatile and non-volatile taste components and sensory taste attributes.

Tomatoes
Tomatoes of *cv.* Tradiro were, based on their colour, harvested at two maturity stages. These tomatoes were stored at four different temperatures, respectively at 3 ^{0}C (chilling injury temperature) , 12 ^{0}C (optimal storage temperature) 20 ^{0}C and 25 ^{0}C , all at a relative humidity of about 90%. During storage, which lasted for about four weeks, samples were withdrawn for further analyses. This analysis comprised:

Non-destructive analysis on individual tomatoes
- Texture (compression) measurements,
- NIR spectroscopy of the intact tomato samples.

Destructive analysis
For each temperature-time combination the homogenate of 20 tomatoes was used for further analysis:
- analysis of the major, non-volatile taste components; sugars and organic acids,
- analysis of the activity of major, texture related enzymes.

On basis of this information matrix two approaches were addressed:

- The development of mathematical, more fundamental models oriented towards the modelling of the underlying processes that cause the observed phenomena (e.g. the temperature dependent change in texture) rather than the modelling of the observed phenomena themselves. The models are based on kinetic mechanisms describing the particular process.
- The development of statistical models, especially focused on the relation between NIR spectra and the temperature dependent change in texture, non-volatile taste components and texture related enzymes.

With reference to the mathematical, more fundamental models the following models were built:
Based on non-destructive measurements:

- a model on Firmness, based on compression measurements
- a model on water loss

Based on destructive measurements:

- a model on the behaviour of PG activity
- a model on the behaviour of PE activity
- a model on the behaviour of β-galactosidase activity

Statistical models build in this project are models :
Based on non-destructive measurements:

- a NIR model predicting the Firmness, based on non-destructive compression measurements
- a NIR model predicting the water loss

Based on destructive measurements:

- NIR models predicting the sugar content (glucose and fructose)
- NIR models predicting the organic acid content (citric and malic acid)
- NIR model predicting the PG activity.
- NIR model with simultaneously predict the PG activity and Firmness

1 MATERIALS

1.1 Apples

1.1.1 Apple variety and storage conditions

Apples (Jonagold, Cox (Orange Pippin)) were received from the VBT or obtained (Elstar) from a commercial storage facility in The Netherlands. These apples, upon arrival at ATO-DLO, were immediately stored under CA-conditions Jonagold, Cox).Starking apples at different degrees of mealiness were from the VBT. The CA-storage conditions are respectively:

Jonagold 1 ^{0}C, CO_2=4.5%, O_2=1.2%,
Cox: 4 ^{0}C, CO_2=0.7%, O_2=1.3%.
Elstar: 4 ^{0}C, CO_2=0.7%, O_2=1.3%.

1.1.2 Development of mealiness in apples.

In order to develop different mealiness levels apples (Cox, Jonagold and Elstar) were stored in perforated plastic bags at 20 ^{0}C. Every fifth day (Cox), or every seventh day (Jonagold, Elstar) a fraction of a specific sub-batch was transferred from CA-conditions into plastic bags. After 20 days there were 5 samples with different mealiness levels for Cox. After 28 days there were 5 samples with different mealiness levels for Jonagold and Elstar.

1.1.3 Experimental design for destructive and non-destructive analyses

Apples with five different mealiness levels were further analysed using:

i) non-destructive techniques
   - Near Infra Red (NIR) spectroscopy

ii) destructive techniques
   - sensory measurements
   - HPLC- analysis; analysis of non- volatile taste components; sugars, and organic acids
   - dry matter content

1.2 Tomatoes

1.2.1 Tomato samples and harvesting

Tomatoes, *cv.* Tradiro, were harvested (KUL) in April 1998, at two colour stages; colour stage 6 and 8. Throughout the rest of this report colour stage 6 will be referred to as "Unripe", colour stage 8 will be referred to as "Ripe".

1.2.2 Storage experiments

Tomatoes were stored at respectively 3 ^{0}C (chilling injury temperature) , 12 ^{0}C (optimal storage temperature) 20 ^{0}C and 25 ^{0}C , all at a relative humidity of about 90%. During storage samples were withdrawn for further analyses. Two designs were applied. One design was used where the samples were put back after analyses (determination of water loss) and one design where the samples were measured and processed for further analyses (rest of the analyses; both destructive and non-destructive).

1.2.3 Experimental design for destructive and non-destructive analyses.

For the destructive and non-destructive analyses the following sampling scheme was applied:

Table 1.1: *Sampling scheme for both "Ripe" and "Unripe" tomatoes*

Time (day)	Storage Temperature 3 ^{0}C	12 ^{0}C	20 ^{0}C	25 ^{0}C
1	+	+	+	+
2		+	+	+
3	+		+	+
6	+	+	+	+
7			+	+
8	+	+	+	
9				+
10	+			
13	+	+	+	+
15	+	+		
20	+	+	+	+
22		+		
29	+	+		

For each temperature-time combination (see Table 1.1) twenty tomatoes were used for analyses. The day after arrival (= day 0), the same analyses were performed as during storage. In total 1440 individual tomatoes were analysed.

1.2.3.1 Non-destructive analysis

The following non- destructive analyses were performed on individual tomatoes:

- Instron measurements (flat plate compression): values determined were slope (N/m), distance (m) and tomato-diameter (m).
- Near Infra Red (NIR) measurements between 1100-2500 nm.

Prior to the non-destructive measurements the tomatoes were accommodated to room temperature for about two hours to avoid temperature effects on the performed measurements.

1.2.3.2 Destructive analysis

After performing the non-destructive analyses one sample was prepared from the twenty individual tomatoes. Of this sample the following parameters were determined:

- dry matter content
- abundant sugars: glucose, fructose, saccharose
- abundant acids: citric acid, fumaric acid, oxalic acid malic acid and pyroglutamic acid
- vitamin c: ascorbic acid and dehydro-ascorbic acid
- protein
- enzymes: pectin methyl esterase, polygalacturonase, β-galactosidase

In total 70 samples were analysed.

1.2.4 Data sets on tomatoes

The following data sets were developed :

I Data set on water loss;

- Two ripeness stages: Ripe and Unripe
- Four storage temperatures
- Eight measuring times
- Twenty tomatoes per ripeness stage and temperature
- In total 160 tomatoes

Transformation of data on water loss: The sampling frequency of the experiment on water loss (see *2.1.3*) was not identical to the sampling frequency such as given in Table 1.1. Estimates for the values on water loss for a given storage day were made by linear intrapolation of the measured water loss (average value of twenty tomatoes) on the nearest by day before and nearest by day after the storage day using the data such as obtained under *2.1.3*.

II Data set on non-destructive measurements

- Two ripeness stages: Ripe and Unripe
- Four storage temperatures
- Measuring times; see Table 1.1
- Data obtained on individual tomatoes
  - compression measurement (slope and distance),
  - NIR spectrum
- In total 1440 individual tomatoes were analysed

III Combined, averaged data-set on destructive and non-destructive measurements

- Two ripeness stages: Ripe and Unripe
- Four storage temperatures
- Measuring times; see Table 1.1
- Data obtained:
  - averaged value for twenty tomatoes per ripeness/storage temperature/storage time combination for both, non-destructive (see 1.2) and destructive (see 2.2) measurements
  - samples of twenty tomatoes per ripeness stage and temperature were analysed; after the non-destructive measurements the tomatoes were processed for further bio/chemical analyses (sugars, organic acids, protein and enzyme activities)
- In total 1600 tomatoes

2 METHODS

2.1 Non-destructive measurements

2.1.1 Near Infra Red (NIR) measurements; apples and tomatoes

NIR measurements (resolution of 4 nm) were performed using a Bran+Luebbe Infra Analyser 500, (PbS detector) and equipped with a fibre optic set up, using IDAS software. The reflection spectra were taken alongside the equator of the fruit.

2.1.2 Compression measurements of tomatoes

Compression measurements were performed using a Universal Testing Machine, Instron 4301. A flat plate compression was applied at a speed of 20 mm/min and a force of 3N. Making use of this set up the diameter (m), the slope (N/m) and the distance (m) were determined given the above mentioned conditions.

2.1.3 Determination of water loss of tomatoes

Twenty tomatoes, either Ripe or Unripe, were stored for thirty days at a temperature of respectively 3, 12, 20 and 25 ^{0}C. At day 0 (upon arrival), day 1, 2, 7, 13, 21 and 30 the weight of all the individual tomatoes was determined. After weighing the tomatoes were put back to their original storage conditions. The loss in weight was assumed to be mainly caused by water loss, ignoring the metabolic processes contribution to the weight loss (not determined). In total 160 individual tomatoes were analysed.

2.2 Destructive measurements

The destructive measurements comprised:

- sensory analysis of apples
- determination of dry matter content of apples and tomatoes
- HPLC analysis of non-volatile taste components (sugars and organic acids) of apples and tomatoes
- enzyme analysis of tomatoes

2.2.1 Sensory analysis

2.2.1.1 Sensory panel

The sensory analytical panel consisted of 20 in-house assessors; 11 men and 9 women, aged between 25-35 years.

2.2.1.2 Sample preparation

Each apple was divided into two pieces from stem-end to calyx-end through the blushside of the apple. Next, these pieces were subsequently divided into three pieces from stem-end to calyx-end.

Afterwards the cores were removed and all pieces were peeled. Samples were presented to the assessors in plastic containers equipped with a four digit code.

2.2.1.3 Method used for measuring

After the training period the assessors evaluated apples using QDA. The sensory attributes were rated on a line scale from 5-95 anchored on both ends from "present slightly" to "present strongly".

2.2.1.4 Data-analysis techniques

The data of the ranking were evaluated according to Newell (1987). For the data obtained with the descriptive profiling Principal Component Analysis (PCA) and analysis of variance (ANOVA; with a confidence level of 95%) were used to examine differences between the products. In addition the statistical packages UNSCRAMBLER
(v. 6.1, CAMO, Norway) and SPSS (v. 6.1.4, The Netherlands) were used to analyse the data. The statistical package SENSTOOLS (v. 2.2, OP&P, The Netherlands) was used to study the assessors behaviour and the correlations between individual scores and mean scores for each descriptor.

2.2.2 Dry matter content of apples and tomatoes

The dry matter content of the samples was determined by drying a known weight of homogenised fresh samples overnight at 70 °C, followed by 3 h at 105 °C. After cooling to room temperature in a dessicator, the samples were weighed again. The dry matter and water content were calculated from the weight difference.

2.2.3 Analysis of sugars and organic acids (non-volatile taste components) of apples and tomatoes

2.2.3.1 Sample preparation of apples

Fresh samples were peeled and cut into slices to obtain ten equal pieces. From the middle part of one red piece and one green piece cubes of approx. 1 cm^3 were taken. These cubes were immediately frozen with liquid nitrogen to avoid enzymatic activity. The samples were stored at -80 ^0C until analysis. Before extraction the frozen samples were mixed with a Moulinex household mixer to obtain a homogeneous sample. All experiments were performed in duplicate.

2.2.3.2 Sample preparation of tomatoes

After the non-destructive analyses (compression and NIR) the set of twenty tomatoes, representing one storage time-temperature combination, was further processed. Each tomato was cut into four and both the locular mass and seeds were removed. Two, non-adjacent quarters were directly frozen into liquid nitrogen prior to further analyses. The other two quarters were discarded.

2.2.3.3 Chemical analyses of sugars and organic acids of apples and tomatoes

Extraction and subsequent HPLC analyses of sugars and organic acids was performed according to Luning et al. (1994).

2.2.4 Enzyme analyses of tomatoes

All handling was performed at 4 ^0C. Ground frozen samples were immersed in 2 M NaCl and homogenised with an Ultraturrax by three bursts of 30 sec. After centrifugation, low molecular mass compounds were removed from the extracts by elution over a prepacked Sephadex G-25 column (Pharmacia PM10). Fractions containing proteins were pooled and assayed for enzyme activities and protein.

The following enzymes, presumably related to the (decrease in) firmness of tomatoes were determined; pectin methyl esterase (PME; EC 3.1.1.11) endo-polygalacturonase (PG; EC 3.2.1.15) and β-galactosidase (EC; 3.2.1.23).

- *PME activity.*
  PME activity in the supernatant was determined using a continuous spectrophotometric assay with bromothymol blue as a pH indicator (Hagerman and Austin 1986),
- *Polygalacturonase (PG) activity.*
  PG activity was determined spectrophotometrically following derivatisation of the reaction product with UV-absorbing 2-cyanoacetamide as is described by Gross (1982). -*β-Galactosidase.*
  The activities of *β*-Galactosidase was analysed using the *β*-D-galacto-pyranoside-*p*-nitrophenyl (SIGMA) as substrate. The reaction mixture consisted of 1.5 ml of 33 mM acetate buffer of optimum pH (pH 3.5 for galactosidase), 50 mM NaCl and 3 mM of the corresponding PNP-derivative. The reaction mixture was incubated at 30 ^{0}C before addition of sample solution. After 20 min incubation at 30 ^{0}C the reaction was terminated by the addition of 1.5 ml of 0.2 M Na_2CO_3. The activity was calculated from the amount of PNP formed using the molar extinction coefficient of PNP at 420 nm (4.8 x 10^3 M^{-1} cm^{-1}).
- *Protein content*
  Protein in the fractions was analysed with the Coomassie Plus Protein Assay Reagent from Pierce (cat nr 23236) using BSA as reference protein.

2.2.5 Modelling

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 analyses was carried out according to (Tijskens *et al.*, 1997p). 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 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.

3 RESULTS ON TOMATOES

3.1 Results on non-destructive analyses: modelling Firmness

3.1.1 Model development on Firmness

Texture and firmness of fruits and vegetables are based on the presence of different chemical components, like pectins in the middle lamellae and the cellulose in the primary cell wall, and on physical items like archestructure and turgor (Tijskens *et al.* 1997). Fruits of tropical and subtropical origin are most often prone to chilling injury (Tijskens *et al.* 1994). Some of these items can deteriorate during storage, some can not. Without chilling injury, the firmness of fruits and vegetables will consequently decay during storage to a certain (predefined) firmness level at infinite storage time. The effect of this chilling injury will most often be visible in a lower firmness level at infinite storage time.
This whole situation can be summarised in the following reaction mechanism:

$$F_w \xrightarrow{k_w} Decay$$
$$F_w \xrightarrow{k_c} Decay \qquad (1)$$
$$F_c \xrightarrow{k_c} Decay$$

where F = the firmness, *Decay* are (unimportant) reaction products, k = reaction rate constant, indices "w" refers to the chemical component that can deteriorate during storage at "normal" temperatures and "c" refers to the chemical component that can deteriorate during storage at "chilling" temperatures.
Based on the fundamental rules of chemical kinetics, the following set of differential equations can be deduced:

$$\frac{dF_w}{dt} = -(k_w + k_c) \cdot F_w$$
$$\frac{dF_c}{dt} = -k_c \cdot F_c \qquad (2)$$

At constant external conditions (like temperature as in the experimental series), an analytical solution can be obtained for both firmness aspects F_w and F_c by solving the set of differential equations. By summing both firmness aspects (F_w and F_c) and adding an unchangeable part (F_{fix}) one obtains:

$$F = F_{w,0} \cdot e^{-(kw+kc)\cdot t} + F_{c,0} \cdot e^{-kc\cdot t} + F_{fix} \qquad (3)$$

All rate constants k_i are modelled and used as depending on temperature according to Arrhenius' law:

$$k_i = k_{i,ref}\, e^{\frac{E_i}{R}\left(\frac{1}{T_{ref}} - \frac{1}{T_{abs}}\right)} \qquad (4)$$

The reference temperature T_{ref} was put to 20 ^{0}C in all analyses.

3.1.2 Raw data on firmness and relations

In the experimental set-up, both the slope and the compression distance were measured. If we idealise the measuring graph to a triangle with height the predefined end-force F_{end}, and the base the compression distance (D), the slope can roughly be approximated by F_{end}/D. This signifies that slope and distance are inversely related: D= F_{end} / Slope. As sensory firmness is mostly connected to tissue breaking force, and the slope always correlates well with the breaking force, the slope was directly related to the develop firmness model (eqn. 3), the distance on the other hand is inversely related to the **same** model (eqn. 5).

$$D = \frac{F_{end}}{F_{w,0} \cdot e^{-(kw+kc)\cdot t} + F_{c,0} \cdot e^{-kc\cdot t} + F_{fix}} \qquad (5)$$

In Figure.3.1 the data for the measured slope of tomatoes for the two harvest maturities stored at 4 different temperatures is shown. As can readily be seen the unripe tomatoes are, as expected, somewhat firmer than the tomatoes that are harvested at a more mature stage. This should be taken into account when analysing the data of both harvest maturities together.
A second effect can directly be taken from Figure 3.1: the tomatoes stored at 3 °C, from both harvest maturities show clearly a decrease toward a lower end-level in firmness.
Even those stored at 12 °C do exhibit to a small extent the same behaviour. This constitutes the part of firmness decay caused by the induced chilling injury. In the model formulation it is covered by the decay of F_c.
The effect of a lower end-value in firmness induced by chilling decay, will be more explicitly expressed in the distance data, by the simple inverse relation as deduced in equation 5. In Figure. 3.2 these data are shown. Again we can see the effect of chilling temperatures as expected in the measured data.

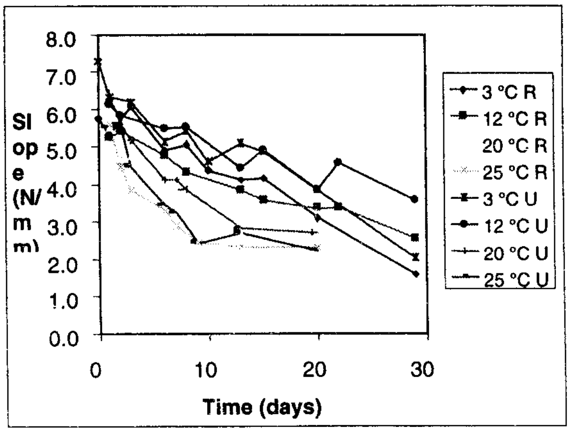


Figure 3.1: Firmness measured as slope for two stages of harvest maturity, stored at 4 different temperatures

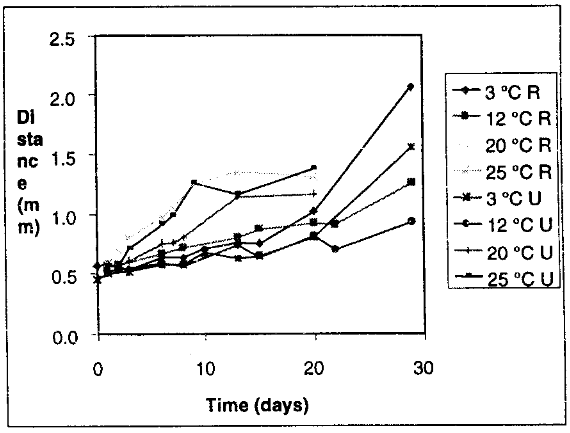


Figure 3.2: Firmness measured as compression distance for two stages of harvest maturity.

3.1.3 Statistical analyses

Based on the developed models, the normalised data (actual individual slopes, or actual individual distances divided by the mean slope respectively the mean distance) were statistically analysed by multiple non-linear regression on slope (equation 3) and compression distance (equation 5) separately, and both combined. The weight was put equal to the inverse of the measured and normalised data to avoid excessive importance of the high data values. For the slope analyses, high values are at the start of storage, for distance analyses they are at the end, and for the combined analyses high values represent both extremities. In Table 3.1 the results of the non-linear regression analyses are shown.
Which of the three analyses should be used for description and prediction cannot be deduced from this data set that is rather limited both on the number of studied temperatures and the number of samples exhibiting chilling injury. It is however, clear that unripe tomatoes have an initial firmness ($F_{w,0}$(unripe)) that is about 24% higher than tomatoes picked one week later ($F_{w,0}$(ripe)). The information on the processes occurring

Table 3.1 *Results of statistical analyses of the normalised data*

	Slope	Distance	Combined
N_{obs}	70	70	140
R^2_{adj}	91.6	86.8	89.9
R^2_{adj} (all data)	89.7	89.7	
F_{fix}	0.2499	0.2533	0.2503
$F_{w,0}$ (unripe)	1.084	1.086	1.115
$F_{w,0}$ (ripe)	0.8394	0.8408	0.8518
$F_{c,0}$	0.2613	0.2620	0.2775
$k_{w,ref}$	0.1294	0.1299	0.1401
E_w/R	9922	9921	9971
$k_{c,ref}$	$4.68\ 10^{-8}$	$3.48\ 10^{-8}$	$9.52\ 10^{-9}$
E_c/R	-63801	-65700	-71793

exclusively during safe, "non-chilling injury" storage (10, 20 and 25 ^{0}C) is estimated in all three analyses as the same. This signifies that sufficient information is contained within the data to estimate the parameters involved ($k_{w,ref}$ and E_w/R). The fixed part of firmness, that cannot be degraded, not even by chilling injury related processes (F_{fix}), is about 24%, while the part that can be degraded by chilling injury related processes ($F_{c,0}$), is about 26%. Two measuring points seem to be outliers to the statistical system: the longest storage time at 3 ^{0}C for both stages of maturity. This indicates that the model not completely (or not at all) covers the occurring chilling injury process. In view of the very complex situation during chilling injury (Tijskens *et al.* 1994), and the very simple (non-dynamic) experimental set-up, this is not at all surprising.
The complete model has however, sufficient descriptive and predictive power to be useful for practical applications.
The 3D behaviour of firmness expressed as slope and as compression distance, simulated based on the estimated parameters of Table 3.1 is shown in Figure 3.3 and Figure. 3.4. The effects of chilling injury on the end-level of firmness are clearly visible in both Figures and the peculiar behaviour of the measured data (Figures. 3.3 and 3.4) are covered by the model.

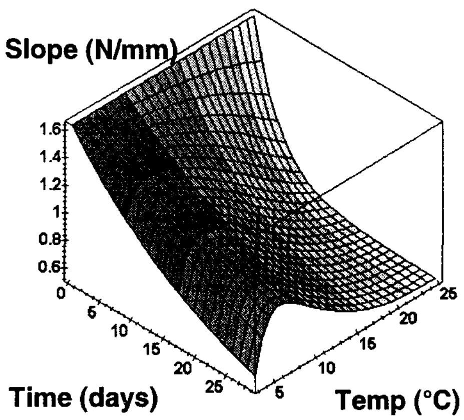


Figure. 3.3: *3D plot of simulated slope*

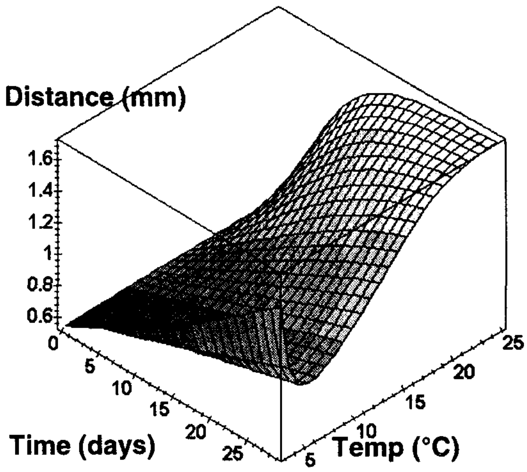


Figure 3.4: *3D plot of simulated compression distance*

3.2 Results on non-destructive analyses: modelling water loss

3.2.1 Raw data on water loss

The weight loss of 20 individual tomatoes during storage was measured and the water loss calculated relative to the weight at (quite arbitrary) day zero: the start of the experiments. What the effect of maturity will be is very difficult to deduce on theoretical grounds, but the general expectation is that the effect is minimal. In Figure 3.5 the average moisture loss is shown.

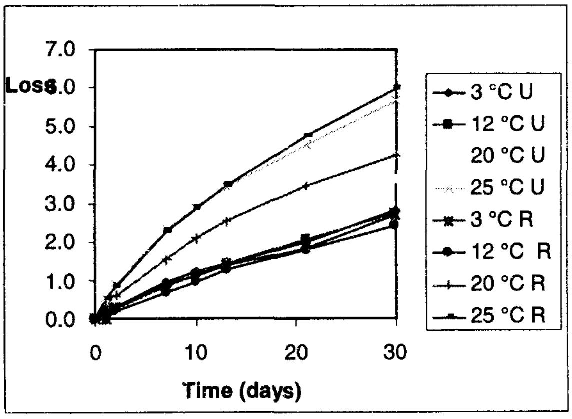


Figure. 3.5: *Water loss in tomatoes at 4 different storage temperatures and two stages of maturity*

The effect of harvest maturity is indeed very small and will be neglected in the further model development and data analyses.
As the Relative Humidity during storage was roughly constant and the same for all 4 temperatures, no effect of RH can be deduced, and RH will not be considered in the model development.

3.2.2 *Preliminary analyses and model development*

At first glance the behaviour of moisture loss in time is exponential towards an end-value: the maximum amount of water a tomato can lose. This maximum potential water loss seems to depend on temperature. In a preliminary analyses applying an exponential decay towards an end-value, with the rate constants again depending on temperature according to Arrhenius' law (equation 4), where ML stands for "moisture loss":

$$ML = ML_{\max} \cdot (1 - e^{-k_{ml} \cdot t}) \qquad (6)$$

The rate constant and its dependence on temperature was estimated in common, the end-values ML_{max} were estimated separately for each storage temperature. The results of this analyses are shown in Table 3.2. The percentage variance explained is extremely high: 99.1%. For the three temperatures not inducing chilling injury a positive relation between ML_{max} and temperature can be observed. At the same time, ML_{max} increases again at the low chilling temperatures. This seems to indicate that the moisture loss is somehow coupled to the relative amount of damage induced by chilling or ripening, in the fruit flesh and / or cell wall structure and material. Most probably this change in end-value of possible moisture loss indicates a conversion of bound water to free water, the latter able to evaporate during storage.

Table 3.2 *Results of preliminary analyses of moisture loss.*

N_{obs}	62
R^2_{adj}	99.1
$k_{ml,ref}$	0.04421
E_{ml}/R	2746.
$ML_{max,3}$	5.128
$ML_{max,12}$	3.881
$ML_{max,20}$	5.416
$ML_{max,25}$	7.218

3.2.3 *Combined analyses and model development*

For the combined analyses, it is assumed that the end value ML_{max} depends on temperature according to two processes, one predominantly occurring at "non-chilling injury" temperatures, one predominant active at chilling temperatures. The energy of activation of the latter process has to be negative, since this process slows down/disappears at increasing temperatures. Although the number of 4 temperatures (one "chilling", three "safe") is far too small for a reliable analyses of the data and a reliable estimation of temperature dependence, it was possible to analyse all data in common with the model as shown in equation 7.

$$\begin{aligned} ML &= ML_{\max} \cdot (1 - e^{-k_{ml} \cdot t}) \\ ML_{\max} &= ML_{\max,eq} + k_{c,i} \end{aligned} \qquad (7)$$

In Table 3.3 the results of the analyses are shown. The percentage variance explained has not changed. This indicates either that the model is correct (for this data set) or that the number of temperatures is too low to estimate two temperature dependencies. The rate constant for moisture loss $k_{ml,ref}$ should depend on the relative humidity. Its energy of activation E_{ml} is rather low which is quite usual for physical processes. The rate constant for the chilling injury induced conversion of bound to free water $k_{ci,ref}$ is at the reference temperature (20 ^{0}C) indeed very low: about half the rate constant of the normal process, with an activation energy E_{ci} that is indeed negative and quite high.

Table 3.3: *Results of combined moisture loss analyses*

N_{obs}	62
R^2_{adj}	99.1
$k_{ml,ref}$	0.04161
E_{ml}/R	3679
$ML_{max,eq}$	5.603
$E_{ml,eq}/R$	4237
$k_{ci,ref}$	0.02343
E_{ci}/R	-17862

In Figure 3.6 the 3D simulation of water loss is shown.

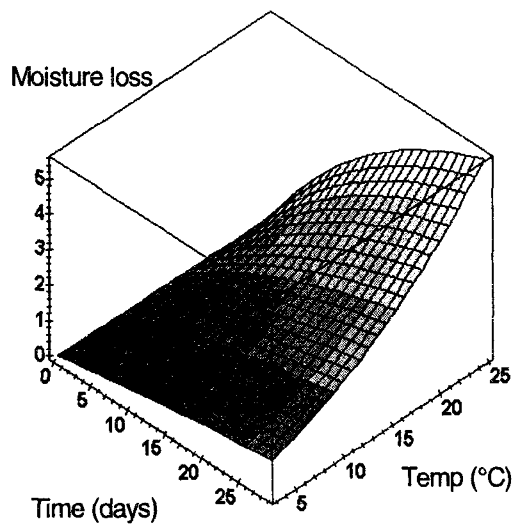


Figure. 3.6: *Simulation of moisture loss*

3.3 Results on non-destructive measurements: NIR prediction

In the previous sections (3.1 and 3.2) emphasis was put on the modelling of (assumed) underlying fundamental process causing the measured changes in compression measurements (slope and distance) and water loss. In this part a "black box" approach is chosen to establish statistical relations between infra red spectra and measured properties, both non-destructive as well as destructive. In this section the focus will be on the non-destructive measurements "slope", "distance" and "water loss". In first instance optimal PLS1 models were made for these three variables with regard to minimal number of Principal Components (PC's), maximum correlation and prediction values and minimal numbers of rejected outliers, taking all the samples into account. Validation ,testing the models to get an estimate of the prediction error in future predictions, was done by full cross validation of the samples.

3.3.1 NIR prediction and validation on non-destructive measurements of the combined, averaged data-set

To make a comparison possible between the physical measurements and (bio)chemical measurements the average values (n=20) per storage time- temperature combination (see Table 1.1) was determined for the variables slope, distance, water loss and the NIR spectra. These average values were used for further statistical analysis
An overview of this analyses is given in Table 3.4.

Table 3.4 *Results of a statistical analysis of the NIR prediction of the "slope", "distance" and "water loss" of all tomato samples (Ripe and Unripe) stored at four different temperatures. Total number of different samples is 70.*

Statistical information	**Variable**					
	Slope		*Distance*		*Water loss*	
	Calibration	Validation	Calibration	Validation	Calibration	Validation
R	0.963	0.881	0.961	0.916	0.965	0.850
RMSEP	$3.36\ 10^{-1}$	$5.88\ 10^{-1}$	$8.35\ 10^{-2}$	$1.12\ 10^{-1}$	$2.52\ 10^{-1}$	$4.61\ 10^{-1}$
Bias	$2.42\ 10^{-8}$	$6.48\ 10^{-3}$	$3.50\ 10^{-5}$	$3.05\ 10^{-3}$	$3.36\ 10^{-7}$	$5.10\ 10^{-3}$
Number of outliers	1		2		3	
Number of PC's	5		7		5	

R; correlation coefficient, RMSEP; root mean square error of prediction

In general terms, the models for "*Slope*", "*Distance*" and "*Water loss*" are reliable models based on both their calibration and validation values. The reliability of the models increase slightly in the range "*Water loss*", "*Slope*", "*Distance*".
The reliability of these three overall models (NIR-prediction models) was tested on several sub-samples, respectively:

- tomatoes stored at 3, 12, 20 and 25^{0}C respectively
- the "Ripe" and "Unripe" tomatoes
- tomatoes stored for less than 8 days
- tomatoes stored for 8 days or more
- all the tomatoes, without any exception

In Tables 3.5, 3.6 and 3.7 the results of this analyses are respectively given for "slope", "distance" and "water loss".

Table 3.5: *NIR prediction model for "slope" tested on several sub-samples*

NIR prediction model for "slope"			
Sample	*R*	*RMSEP*	*Bias*
Stored at 3°C	0.914	$5.15\ 10^{-1}$	$8.77\ 10^{-2}$
Stored at 12°C	0.949	$3.51\ 10^{-1}$	$1.13\ 10^{-1}$
Stored at 20°C	0.966	$3.01\ 10^{-1}$	$5.21\ 10^{-2}$
Stored at 25°C	0.961	$3.85\ 10^{-1}$	$2.09\ 10^{-1}$
Ripe	0.928	$4.37\ 10^{-1}$	$1.09\ 10^{-1}$
Unripe	0.963	$3.53\ 10^{-1}$	$5.72\ 10^{-2}$
Short storage (< 8 days)	0.908	$4.49\ 10^{-1}$	$8.42\ 10^{-2}$
Long storage (≥ 8 days)	0.944	$3.45\ 10^{-1}$	$2.89\ 10^{-2}$
All samples	0.948	$3.99\ 10^{-1}$	$2.60\ 10^{-2}$

Table 3.6: *NIR prediction model for "distance" tested on several sub-samples*

NIR prediction model for "distance"			
Sample	*R*	*RMSEP*	*Bias*
Stored at 3°C	0.989	$5.89\ 10^{-2}$	$1.73\ 10^{-4}$
Stored at 12°C	0.906	$8.30\ 10^{-2}$	$7.79\ 10^{-3}$
Stored at 20°C	0.919	$1.00\ 10^{-1}$	$2.34 10^{-2}$
Stored at 25°C	0.952	$9.37\ 10^{-2}$	$2.81\ 10^{-2}$
Ripe	0.940	$1.08\ 10^{-1}$	$8.82\ 10^{-3}$
Unripe	0.946	$9.03\ 10^{-2}$	$1.00\ 10^{-2}$
Short storage (< 8 days)	0.907	$7.52\ 10^{-2}$	$1.54\ 10^{-2}$
Long storage (≥ 8 days)	0.932	$1.18\ 10^{-1}$	$3.77\ 10^{-3}$
All samples	0.944	$9.97 10^{-2}$	$9.43\ 10^{-3}$

Table 3.7: *NIR prediction model for "water loss" tested on several sub-samples*

NIR prediction model for "water loss"			
Sample	*R*	*RMSEP*	*Bias*
Stored at 3°C	0.975	$2.05\ 10^{-1}$	$1.72\ 10^{-2}$
Stored at 12°C	0.944	$2.87\ 10^{-1}$	$8.95\ 10^{-1}$
Stored at 20°C	0.969	$2.26\ 10^{-1}$	$3.54\ 10^{-2}$
Stored at 25°C	0.967	$2.97\ 10^{-1}$	$1.44\ 10^{-1}$
Ripe	0.900	$5.02\ 10^{-1}$	$4.03\ 10^{-2}$
Unripe	0.967	$2.41\ 10^{-1}$	$6.70\ 10^{-2}$
Short storage (< 8 days)	0.923	$2.61\ 10^{-1}$	$2.18\ 10^{-2}$
Long storage (≥ 8 days)	0.946	$2.42\ 10^{-1}$	$2.25\ 10^{-2}$
All samples	0.893	$4.87\ 10^{-1}$	$4.68\ 10^{-2}$

From the results presented in Tables 3.5 - 3.7 it is obvious that the overall NIR models for these three variables are capable to describe the systematic variations brought about by storage temperature an storage time, irrespective of the maturity stage of these tomatoes. In other words these systematic variations are contained in and described by the NIR models for the three variables analysed.

In conclusion:
NIR prediction models were made for the variables "slope", "distance" and "water loss". Based on the values of the statistical analysis presented in Table 3.4 it can be concluded that the reliability of these models is adequate. The reliability of the models increase in the range "water loss", "slope", "distance". With regard to the NIR model on "water loss" it has to be realised firstly that spectral

information was obtained on different tomato samples than the tomato samples which were actually used for water loss determinations and secondly that some data on water loss were generated by linear intrapolation. These aspects might add to an increase in variance causing the value for the validation to decrease.
Altogether, it is obvious that the overall NIR models for these three variables such as presented in Table 3.4 are capable to describe the systematic variations brought about by storage temperature an storage time, irrespective of the maturity stage of these tomatoes. In other words these systematic variations are contained in and described by the NIR models for the three variables analysed.

3.4 Results on destructive analyses: models on enzyme behaviour in tomatoes

3.4.1 Modelling PG activity

3.4.1.1 Raw data and Model development

For PG in peaches it was found (Tijskens *et al.* 1998) that a conversion exists between a non-active precursor and an active enzyme configuration. In peaches, that active configuration is susceptible to deterioration by senescence. In tomatoes the conversion to active configuration indeed seems to exists, the deterioration by senescence however, seems to be absent (see Fig. 3.7)
What is overwhelmingly present is a shift in level of activity from the unripe (high) to the ripe (low) tomatoes. The kinetics themselves seem not to be affected by the maturity stage. As the deterioration reaction seems to be absent, the occurring kinetics can be described by the following reaction mechanism:

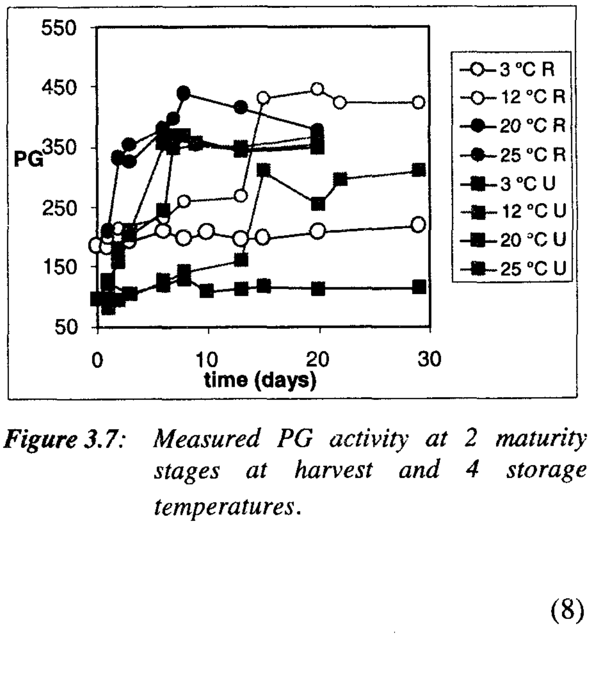


Figure 3.7: *Measured PG activity at 2 maturity stages at harvest and 4 storage temperatures.*

$$PG_{pre} \xrightarrow{k_c} PG \quad (8)$$

From this mechanism the differential equations can be deduced based on the fundamental rules of chemical kinetics:

$$\begin{aligned} \frac{dPG}{dt} &= k_c \cdot PG_{pre} \\ \frac{dPG_{pre}}{dt} &= -k_c \cdot PG_{pre} \end{aligned} \quad (9)$$

At constant external conditions as used in these experiments (constant storage temperatures), and taking an fixed end-value into account, the analytical solution for this set of differential equations is:

$$PG = PG_{pre} \cdot (1 - e^{-k_c \cdot t}) + PG_0 + PG_{fix} \quad (10)$$

In these equations, PG stands for the activity of polygalacturonase, t for the time of storage, k for the reaction rate constant, the indices 0 is initial state, pre is precursor, c is conversion and fix is fixed end-value.
All rate constants k_i are modelled and used as depending on temperature according to Arrhenius' law (eqn. 4) The reference temperature T_{ref} was put to 20 ^{0}C in all analyses.

3.4.1.2 Statistical analyses

As can be taken from the measured data, PG_{fix} will depend on the maturity stage, while PG_{pre} and PG_0 will most probably be independent of the maturity stage.
Based upon the developed model, the data of PG activity were analysed as a function of time (eq.3) and as function of temperature (eq.4) simultaneously with non-linear regression. In Table 3.8 the results are shown for the analyses were all parameters were estimated in common for all temperatures and harvest maturity stage, except for the fixed end-value of PG that was estimated separately for the two stages of harvest maturity. The percentage variance account for (R^2_{adj}) is acceptably high, the standard errors of estimate are relatively low. All parameters estimated have a value within the range normally expected. No effect seems to exist in the behaviour of PG activity at the lower temperatures were chilling injury does occur in tomatoes (3 °C). From this fact we already can (possibly) conclude that the action of PG is not responsible for the extra decrease in firmness and increase in water loss due to occurring chilling injury. At least one more process or enzyme has to be involved.

Table 3.8 *Results of regression analyses for PG in tomatoes*

	estimate	s.e.
PG_{pre}	260.4	16.1
$PG_{fix,U}$	72.5	13.3
$PG_{fix,R}$	162.2	13.3
PG_0	0	fixed
$k_{PG,ref}$	0.2397	0.0396
E_{PG}/R	12801.	1070.
N_{obs}	70	
R^2_{adj}	84.8	
T_{ref}	20	

In Figs. 3.8 and 3.9 the behaviour of PG activity simulated based on the parameters of Table 3.8; (lines) and the measured values (symbols) are shown for the ripe and unripe maturity stage. The data fit reasonably well the measured data, especially for the ripe stage. In this figure (Fig. 3.8) it now also becomes evident that a certain decrease in activity at higher temperatures (25 °C) is present (as in the peaches: Tijskens et al. 1998). The decease is however too small and the data too scattered to include this effect in the model.

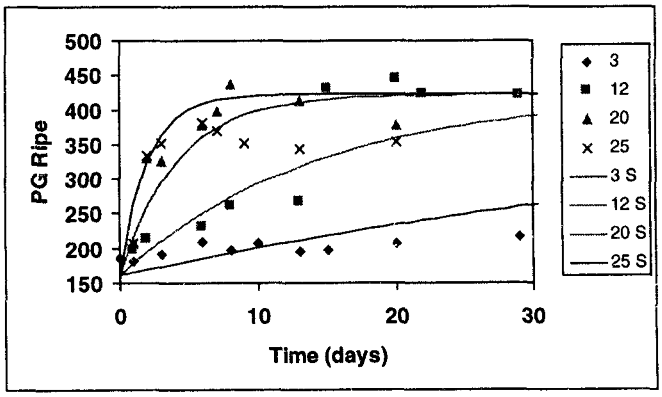


Figure 3.8: *Measured and simulated PG activity for the ripe maturity stage*

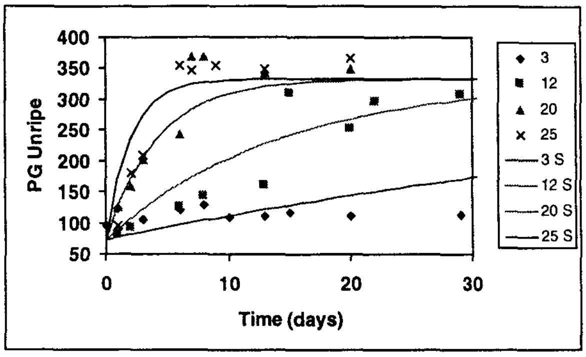


Figure 3.9: *Measured and simulated PG activity for the unripe maturity stage*

3.4.2 Modelling PE activity

3.4.2.1 Raw data and Model development

At first glance, the activity of PE in tomatoes do behave quite normally according an exponential decay (Figure 3.10). This behaviour is not in contradiction with the behaviour during blanching observed in peaches (Tijskens *et al.* 1999) and in carrots and potatoes (Tijskens *et al.* 1997).

The two configurations (bound and soluble) are not found in this temperature range for tomato storage, as there seems not to exist an increase in activity. There is, however, a clear difference between the overall level of the two maturity stages: in ripe tomatoes the activity of PE is about 15 units higher than in unripe tomatoes. This seems to contradict the behaviour measured in these batches of tomatoes: activity decays with time, but in ripening at the plant, activity increases in time. So there is clearly a major effect of the pre-harvest situation on the post-harvest behaviour.

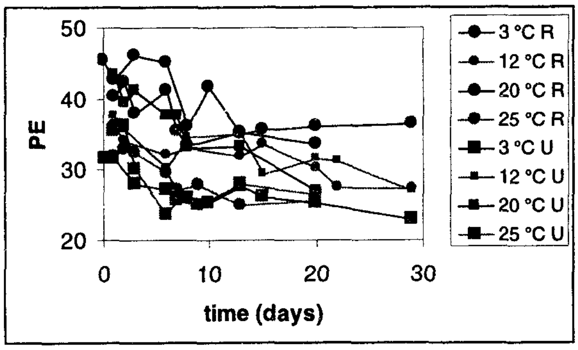


Figure 3.10: *Measured PE activity in tomatoes*

The mechanism that could describe the observed behaviour is a very simple first order decay reaction:

$$PE \xrightarrow{k_d} decay \tag{11}$$

The differential equation is given in equation 6, the analytical solution at constant external conditions (constant temperature) in equation 7.

$$\frac{dPE}{dT} = -k_d \cdot PE \tag{12}$$

$$PE = PE_0 \cdot e^{-kdt} + PE_{fix} \tag{13}$$

In these equations (eqn. 11 to 13) PE signifies the activity, k is reaction rate constant, t is time of storage, indices d is decay, 0 is initially present variable part and fix is invariable fixed part of the activity. The reaction rate constant again depends on temperature according to Arrhenius law (equation 4).

3.4.2.2 *Statistical analyses*

The behaviour of PE activity seems to be quite different for the two stages of maturity. The statistical analyses was conducted for the two stages separately.

The end-value of PE activity at all four temperatures is more or less the same while the variable part seems to be different. This difference in initial condition for the determination of PE activity, is known to be different on a day-to-day basis (Tijskens *et al.* 1997,1999). So, in the statistical non-linear regression analyses, the variable activity (PE_0) is estimated separately, the invariable activity (PE_{fix}) is estimated in common for all temperatures but for each stage of maturity separately. In Table 3.9 the results of the non-linear regression analyses are shown.

Table 3.9: *Results of statistical analyses of PE activity*

	Unripe	Ripe
$PE_{0,3}$	4.65	25.89
$PE_{0,12}$	13.67	17.6
$PE_{0,20}$	22.17	23.26
$PE_{0,25}$	12.73	13.6
Pefix	23.14	18.48
$k_{d,ref}$	0.0827	0.0299
E_d/R	9459.	2286.
R^2_{adj}	77.9	83.9
N_{obs}	35	35
T_{ref}	20	

There is a striking difference in kinetic parameters ($k_{d,ref}$ and E_d) between the two maturity stages. Apparently the decay of PE activity is in itself occurring due to some enzymatic reaction, where the amount of that deterioration depends on the maturity. It is of course not possible to reveal that behaviour based on this data set. A comparable large difference exists in the variable part of the activity at 3 ^{0}C in the unripe stage. It is difficult to accept this is a fundamental and structural difference. Most probably this discrepancy is due to at random differences in the batches of tomatoes used. In Figures 3.11 and 3.12 the behaviour of PE activity simulated based on the parameters of Table 3.8; (lines) and the measured values (symbols) are shown for the ripe and unripe maturity stage.

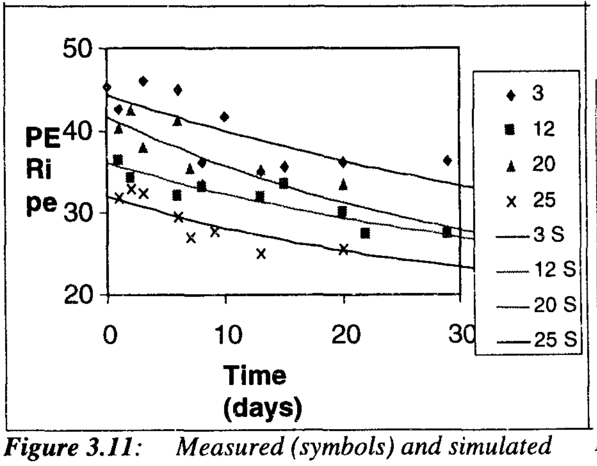


Figure 3.11: *Measured (symbols) and simulated (lines) behaviour of PE at 4 temperatures, for the ripe maturity stage.*

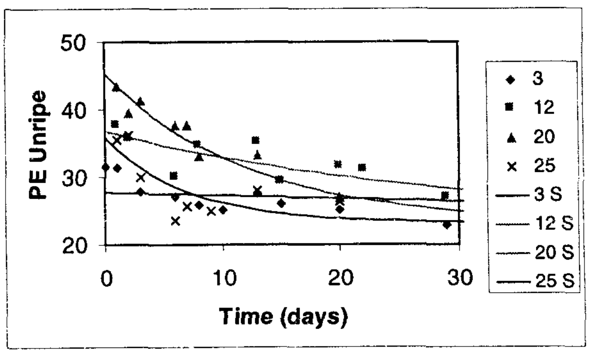


Figure. 3.12: *Measured (symbols) and simulated (lines) behaviour of PE at 4 temperatures, for the unripe maturity stage.*

3.4.3 Modelling β-galactosidase activity

3.4.3.1 Raw data and Model development

The activity of β-galactosidase (GAL) measured in tomatoes clearly show a first increase followed by a decrease during storage at all temperatures (see Figures 3.13 and 3.14). The effect of temperatures causing chilling injury (3 ^{0}C) does not obey the Arrhenius law. The temperature effect resembles more the behaviour at 20 ^{0}C (see Figure 3.14). The deterioration of GAL seems to continue until the same fixed end-value. Apparently the normal turnover, as found for PG in peaches (Tijskens *et al.* 1997), is also valid and occurring for this enzyme.

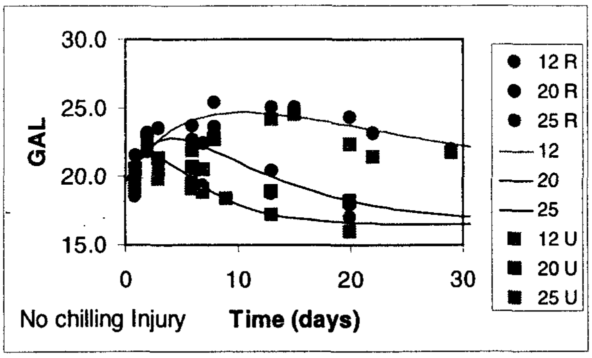


Figure 3.13: *Measured (symbols) and simulated (lines) behaviour of GAL at 3 non-chilling temperatures.*

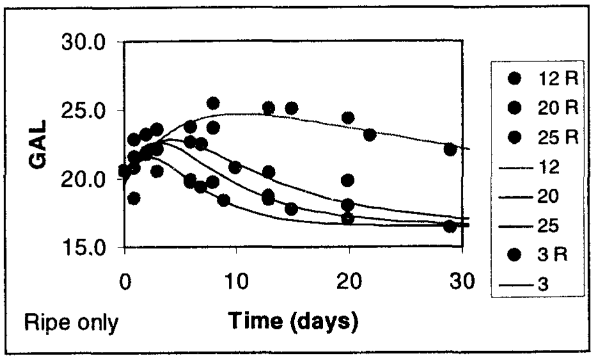


Figure 3.14: *Measured (symbols) and simulated (lines) behaviour of GAL (Ripe maturity) including chilling temperature.*

Without the effect of chilling injury taken into account, the behaviour could be represented by the following reaction mechanism:

$$
\begin{aligned}
&GAL_{pre} \xrightarrow{k_c} GAL \\
&GAL \xrightarrow{k_d} decay
\end{aligned}
\qquad (14)
$$

The corresponding differential equations are given in equation 15:

$$
\begin{aligned}
\frac{dGAL}{dt} &= k_c \cdot GAL_{pre} - k_d \cdot GAL \\
\frac{dGAL_{pre}}{dt} &= -k_c \cdot GAL_{pre}
\end{aligned}
\qquad (15)
$$

At constant external conditions as used in these experiments (constant storage temperatures), and taking a fixed end-value into account, the analytical solution for this set of differential equations is:

$$GAL = GAL_0 \cdot e^{-k_d \cdot t} + \frac{GAL_{pre,0} \cdot \left(e^{-k_c \cdot t} - e^{-k_d \cdot t}\right)}{\left(k_d - k_c\right)} + GAL_{fix} \qquad (16)$$

Both reaction rate constants again depend on temperature according to Arrhenius law (equation 4).

3.4.3.2 Statistical analyses

As the initial activity of GAL seems to be different for the two stages of maturity at harvest, this parameters was estimated separate for the two stages of maturity, all other parameters were estimated in common. All the data of the non-chilling temperature of both stages of maturity were analysed in their entirety with non-linear regression analyses using the develop model (equations 15 and 16).

In Table 3.10 the obtained parameters estimates are shown. The percentage variance accounted for and obtained values of the parameters and standard error of estimates, seem to be quite reasonable, taken the small number of observation and the somewhat erratic behaviour of the measurements into account. The majority of activity of GAL is to be found in the invariable part of the enzyme (GAL_{fix}). The small value of the energy of activation for the conversion reaction E_c relatively to that for the denaturation reaction (E_d) indicates that only at low temperatures and only for a short time an increase in activity can be observed.

Table 3.10: *Results of statistical analyses of GAL activity*

	Estimate	s.e.
$Gal_{0,U}$	1.77	1.18
$Gal_{0,R}$	3.12	1.19
$Gal_{pre,0}$	7.81	1.91
Gal_{fix}	16.519	0.668
$k_{c,ref}$	0.308	0.134
E_c/R	4701.	2039.
$k_{d,ref}$	0.1082	0.0398
E_d/R	15453.	2330.
R^2_{adj}	82.1	
N_{obs}	50	
T_{ref}	20	

The simulated behaviour of GAL activity is shown in Figures 3.13 and 3.14. This also indicates the reasonable fit of the model to the data. The behaviour at 3 ^{0}C is not included in the analyses. Based on visual assessment of that behaviour the model could be extended (but not calibrated) by including in the conversion AND in the denaturation an extra process that becomes more active at lower temperatures. In the analytical solution all occurrences of k_c and k_d have to be replaced by:

$$\begin{aligned} k_c &= k_{c,c} + k_{c,h} \\ k_d &= k_{d,c} + k_{d,h} \end{aligned} \qquad (17)$$

By visual assessment, the values of $k_{c,c}$ and $k_{d,c}$ were given the value of 0.3 and .15 respectively. The values of $k_{d,h}$ and $k_{c,h}$ were used as calculated (see Table 13) The results for the ripe maturity stage are shown in Figure 3.14. The data set is far too small and covers too few temperatures, especially those temperatures that induce chilling injury, to calibrate the extended model with non-linear regression analyses.

3.4.4 *Conclusions on modelling enzyme activities*

- All enzymes studied comply with the generic model GESSI as developed in previous enzyme research (Tijskens *et al.* 1998b).
- More fundamental oriented models are really quite suiTable for statistical analyses of experimental data.
- The behaviour of PG and GAL are affected by the stage of maturity, and hence by the pre-harvest conditions. PE seems not to be affected.
- GAL is the only enzyme studied that responds to chilling injury, and hence physical and/or chemical damage.
- All three enzymes exhibit an end-value at infinite time. What the meaning is of this end-value in systems that include deterioration is not clear. It could be an equilibrium level, an always-present activity, or an artefact.
- Considering chilling injury, one has to realise that the experimental set-up to study this phenomenon is very simple and does not comprise dynamic situation (no warm post storage treatment). As a consequence, the models developed so far do not cover these dynamic situations. A more extended model on chilling injury is described by Tijskens *et al.* (1994).

3.5 Results on destructive analyses: NIR prediction of sugars, organic acids and enzymes

NIR-spectra were obtained from intact tomatoes (see 1.2.3.1). Sugars, organic acids and enzyme activities were determined on homogenates (see 1.2.3.2).

3.5.1 *Sugars*

The most abundant sugars determined in tomatoes are glucose and fructose. No saccharose could be determined in the tomato fruits. The change in the amount of glucose of "ripe" and "unripe" tomatoes is respectively shown in Figures 3.15A and B; the change in the amount of fructose of "ripe" and "unripe" tomatoes in Figures 3.16.A and B. Both for glucose and fructose a small but steady decrease in amount is observed upon storage.

The results of the statistical analysis for the NIR predication and validation models for glucose and fructose are shown in Table 3.11

Table 3.11: *Results of a statistical analysis of the NIR prediction of the glucose, fructose and "glucose+fructose" content of all tomato samples (Ripe and Unripe) stored at four different temperatures. Total number of different samples is 70.*

Statistical information	**Variable**					
	Glucose		*Fructose*		*Glucose+Fructose*	
	Calibration	Validation	Calibration	Validation	Calibration	Validation
R	0.990	0.850	0.916	0.735	0.989	0.837
RMSEP	$1.40\ 10^{-1}$	$5.31\ 10^{-1}$	$2.90\ 10^{-1}$	$4.95\ 10^{-1}$	$2.48\ 10^{-1}$	$9.32\ 10^{-1}$
Bias	$1.85\ 10^{-4}$	$3.04\ 10^{-3}$	$1.05\ 10^{-4}$	$6.06\ 10^{-3}$	$7.10\ 10^{-5}$	$3.15\ 10^{-2}$
Number of outliers	1		4		3	
Number of PC's	12		8		12	

R; correlation coefficient, RMSEP; root mean square error of prediction

The results of the analysis given in Table 3.11 indicate that bot for glucose and for the sum of "glucose + fructose" reliable NIR prediction models could be made.
However for the variable fructose, despite the reasonable value for the calibration , the value for the validation was low. For this reason it can be concluded that for fructose alone no reliable NIR prediction model can be made.
The NIR models for "glucose" and "glucose + fructose" were used to test their predictive power on the several tomato sub-samples. The result of this analyses is shown in Tables 3.12 and 3.13

Table 3.12: *NIR prediction model for "glucose" tested on several sub-samples*

NIR prediction model for "glucose"			
Sample	*R*	*RMSEP*	*Bias*
Stored at 3°C	0.937	$2.85\ 10^{-1}$	$4.65\ 10^{-2}$
Stored at 12°C	0.996	$1.06\ 10^{-1}$	$3.51\ 10^{-3}$
Stored at 20°C	0.985	$1.62\ 10^{-1}$	$2.57\ 10^{-2}$
Stored at 25°C	0.988	$1.56\ 10^{-1}$	$3.20\ 10^{-2}$
Ripe	0.990	$1.37\ 10^{-1}$	$1.16\ 10^{-2}$
Unripe	0.974	$2.33\ 10^{-1}$	$1.95\ 10^{-2}$
Short storage (< 8 days)	0.935	$2.31\ 10^{-1}$	$5.31\ 10^{-1}$
Long storage (≥ 8 days)	0.988	$1.40\ 10^{-1}$	$1.99\ 10^{-1}$
All samples	0.983	$1.90\ 10^{-1}$	$1.55\ 10^{-1}$

Table 3.13: *NIR prediction model for "glucose+fructose" tested on several Sub - samples*

NIR prediction model for "glucose + fructose"			
Sample	*R*	*RMSEP*	*Bias*
Stored at 3°C	0.986	$2.31\ 10^{-1}$	$1.66\ 10^{-2}$
Stored at 12°C	0.956	$5.54\ 10^{-1}$	$1.25\ 10^{-1}$
Stored at 20°C	0.975	$3.17\ 10^{-1}$	$6.33\ 10^{-2}$
Stored at 25°C	0.987	$2.61\ 10^{-1}$	$2.63\ 10^{-2}$
Ripe	0.958	$4.59\ 10^{-1}$	$9.39\ 10^{-2}$
Unripe	0.987	$2.51\ 10^{-1}$	$1.01\ 10^{-2}$
Short storage (< 8 days)	0.976	$2.64\ 10^{-1}$	$2.00\ 10^{-2}$
Long storage (≥ 8 days)	0.967	$4.44\ 10^{-1}$	$4.44\ 10^{-1}$
All samples	0.948	$5.38\ 10^{-1}$	$1.00\ 10^{-1}$

The result of these analyses, given in Tables 3.12 and 3.13 show, that NIR is capable to predict both the amount of glucose as well as the sum of the amount of glucose and fructose.
As indicated above, no suitable NIR model could be made to predict the amount of fructose, however, a reasonable NIR prediction could be made for the sum of the amount of glucose and fructose. As shown in Figures 3.15 and 3.16 the amounts of glucose and fructose are about identical; the amount of fructose is, on average, 10% higher than the amount of glucose. In other words the amount of "glucose + fructose" is about twice the amount of glucose. If, in the prediction of the sum of glucose plus fructose, the fructose would effectively be replaced by glucose, the slope of the prediction for "glucose + fructose" would be about 2 using the glucose prediction model. At the other hand the consequence would also be that under this assumption, the slope of the prediction for glucose would be about 0.5 upon predicting the amount of glucose on basis of the "glucose + fructose" prediction model. This was tested. The result is that in taking the NIR prediction model for glucose to predict the amount of "glucose + fructose" the slope was 1.6 (R=0.94). Taking the NIR prediction model for "glucose + fructose" to predict the amount of glucose the slope was 0.55 (R=0.91), to predict the amount of fructose the slope was 0.40 (R=0.89). This altogether suggests, that for the design of the NIR prediction model for "glucose + fructose" the amount of glucose was "multiplied" with a factor with a value ranging between

1<factor< 2 and the amount of fructose was "multiplied" with another factor ranging between 0<another factor<1. In other words the NIR prediction models contain more relevant information on glucose than on fructose.

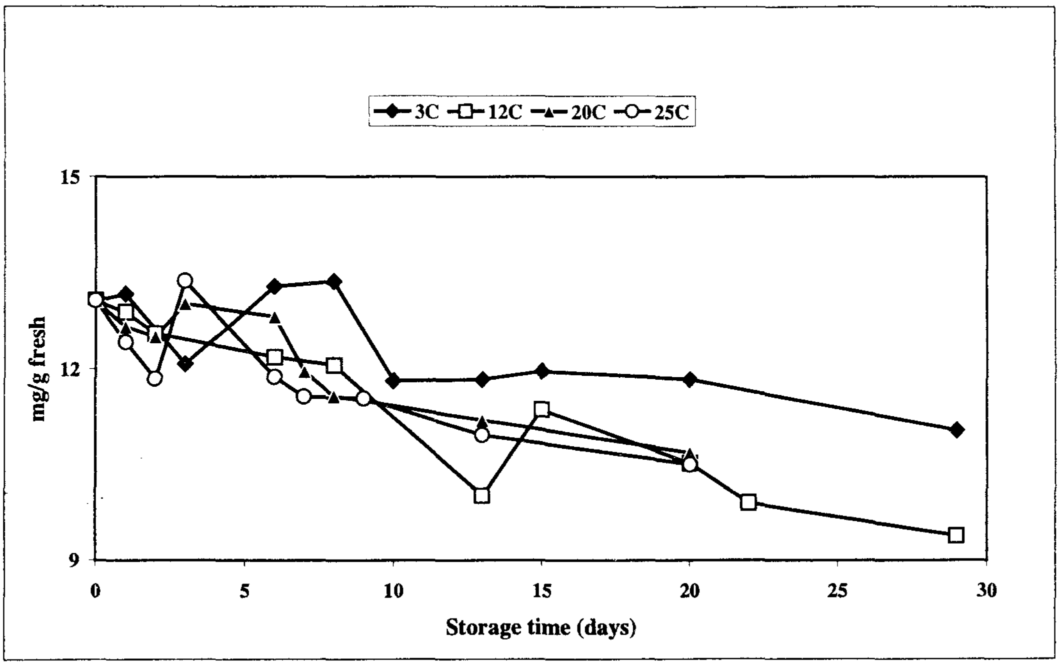


Figure 3.15A: *Amount of glucose of "UNRIPE" tomatoes stored at four different temperatures*

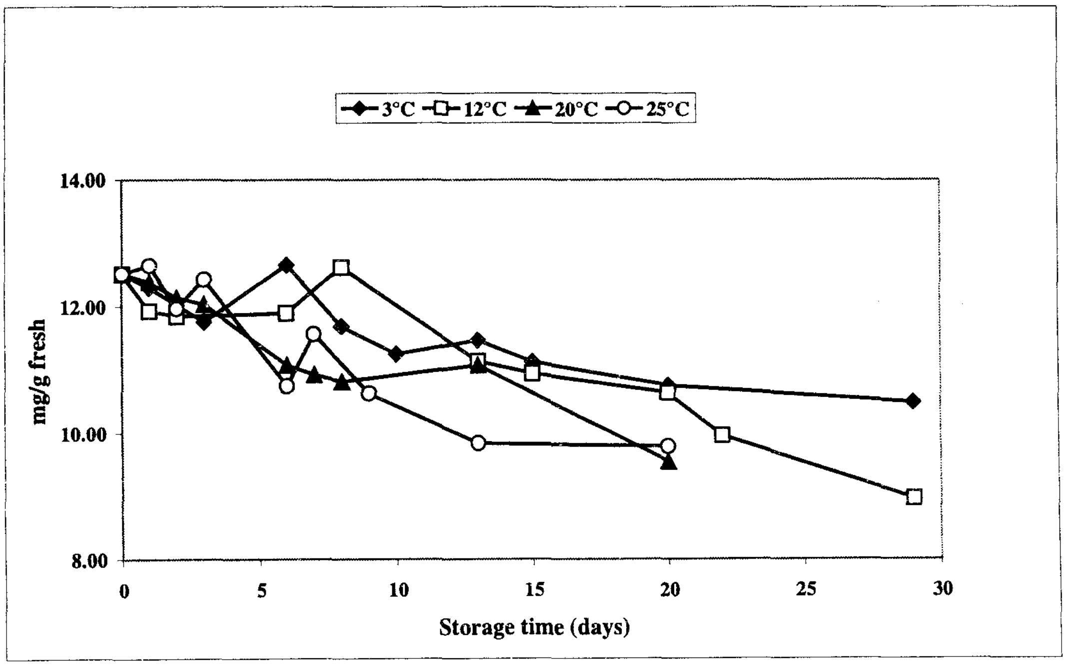


Figure 3.15B: *Amount of glucose of "RIPE" tomatoes stored at four different temperatures*

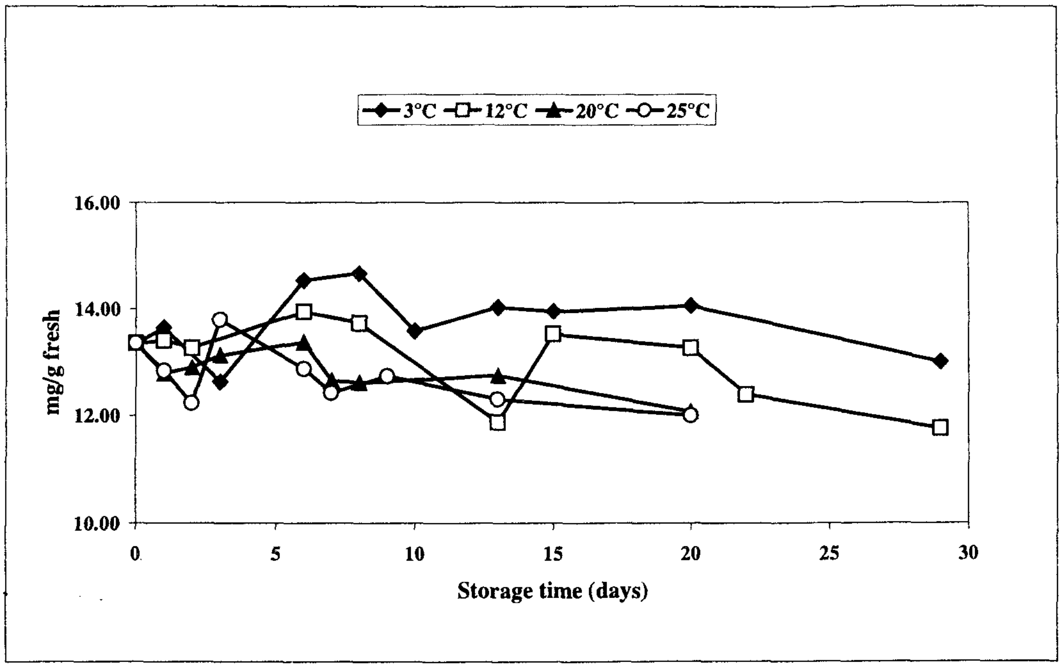


Figure 3.16A: *Amount of fructose of "UNRIPE" tomatoes stored at four different temperatures*

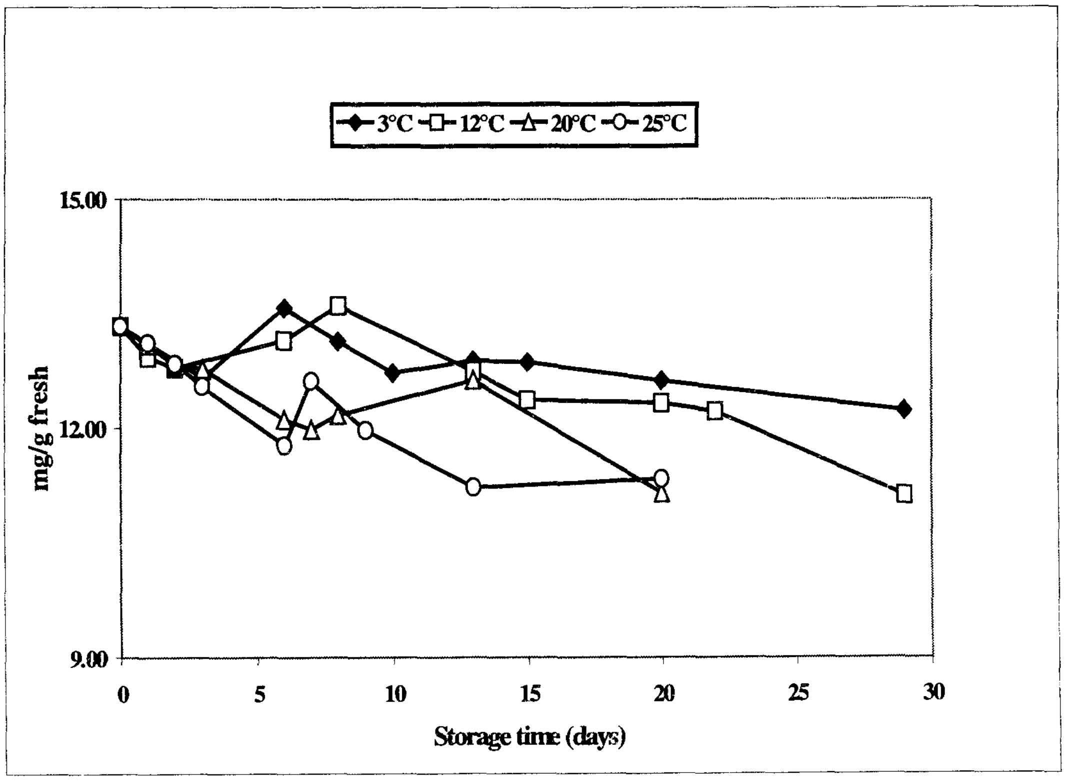


Figure 3.16B: *Amount of fructose of "RIPE" tomatoes stored at four different temperatures*

3.5.2 Organic acids

The amount of citric-, oxalic-, malic-, pyroglutaminic-, and ascorbic acid were determined in all the tomato samples. With regard to oxalic acid (Figure 3.17), ascorbic acid (Figure 3.18) and pyroglutamic acid (Figure 3.19) no proper correlations could be established between the NIR spectra and the amount of these acids.
The most obvious reason for the fact that no proper NIR prediction models could be made for oxalic acid. ascorbic acid and pyroglutamic acid is their relatively low concentration. A general rule for NIR spectroscopy to predict a chemical compound in a matrix is that the lower detection limit of this compound ranges between 0.5-1% (w/w). Obviously the amount of neither oxalic acid, nor ascorbic acid, nor pyroglutamic acid meets this basic requirement.
The more abundant organic acids are citric- and malic acid. In Figure 3.20 the amount of citric acid of "Unripe" tomatoes (Figure 3.20A) and of "Ripe" tomatoes (Figure 3.20B), stored at four different temperatures is shown. In Figure 3.21 the amount of malic acid of "Unripe" (Figure 3.21A) and "Ripe"(Figure 3.21B) tomatoes, stored at four different temperatures is shown.
For both citric acid and malic acid, the predictive power of NIR was estimated. The results of this analyses are shown in Table 3.14.

Table 3.14 *Results of a statistical analysis of the NIR prediction of the citric acid and malic acid content of all tomato samples (Ripe and Unripe) stored at four different temperatures. Total number of different samples is 70.*

Statistical information	**Parameter**			
	Citric acid		*Malic acid*	
	Calibration	Validation	Calibration	Validation
R	0.943	0.844	0.928	0.853
RMSEP	$1.41\ 10^{-1}$	$2.27\ 10^{-1}$	$3.65\ 10^{-2}$	$5.14\ 10^{-2}$
Bias	$1.10\ 10^{-5}$	$9.24\ 10^{-4}$	$8.70\ 10^{-7}$	$9.81\ 10^{-4}$
Number of outliers	6		5	
Number of PC's	8		7	

R; correlation coefficient, RMSEP; root mean standard error of prediction

The predictive power and validity of the NIR models for citric acid and malic acid are slightly less than the predictive and validity of the NIR models for glucose and "glucose+fructose" (see Table 3.11). The most obvious reason for this is the lower amounts of these acids in the tomato fruits as compared to glucose.

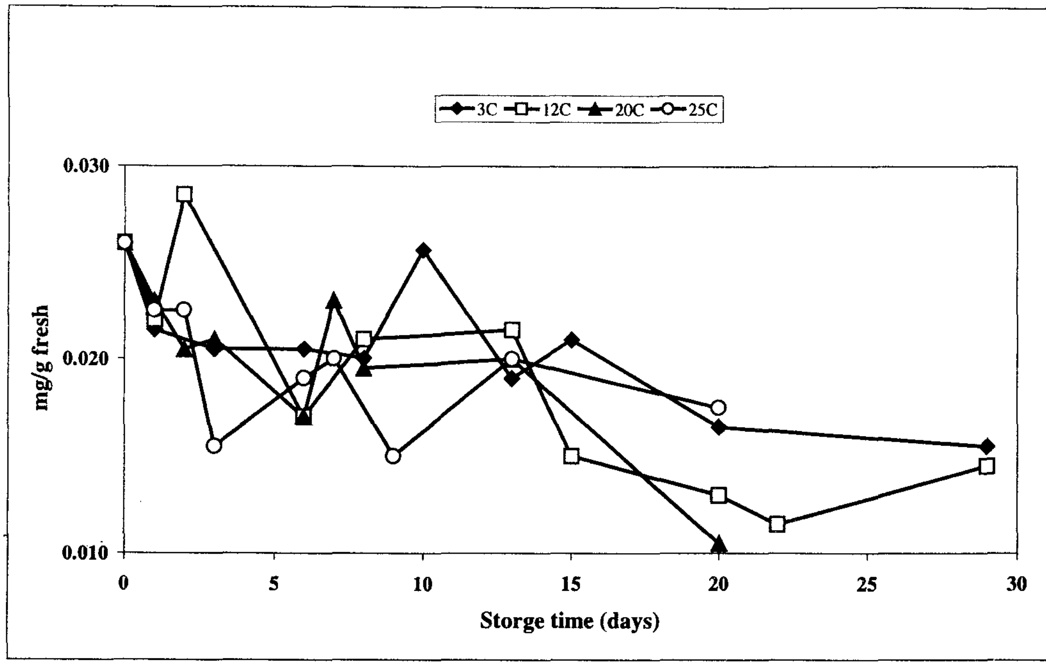


Figure 3.17A: *The amount of oxalic acid of "UNRIPE" tomatoes stored at four different temperatures*

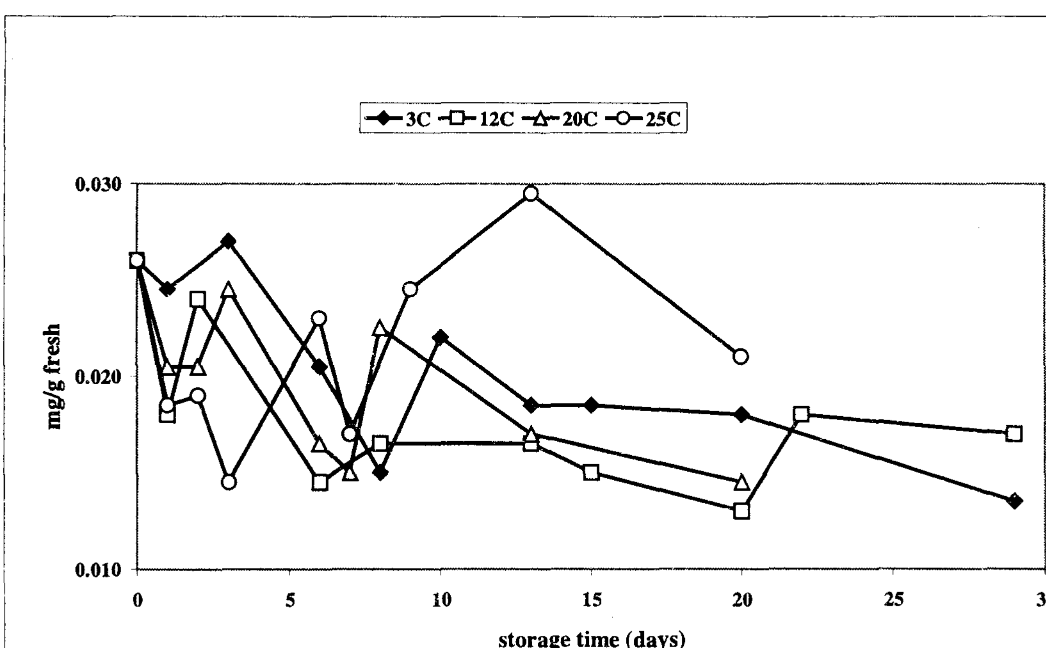


Figure 3.17B: *The amount of oxalic acid of "RIPE" tomatoes stored at four different temperatures*

In Figure 3.18 the amount of ascorbic acid of "Unripe" tomatoes (Figure. 3.18A) and of "Ripe" tomatoes (Figure 3.18B) stored at four different temperatures is given.

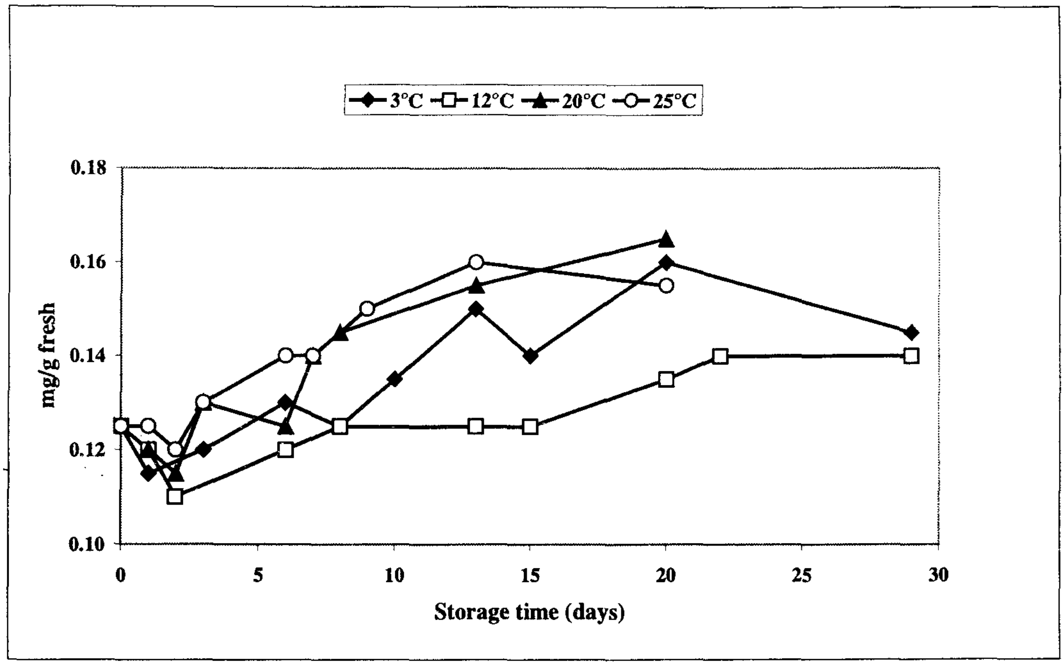


Figure 3.18A: *The amount of ascorbic acid of "UNRIPE" tomatoes stored at four different temperatures.*

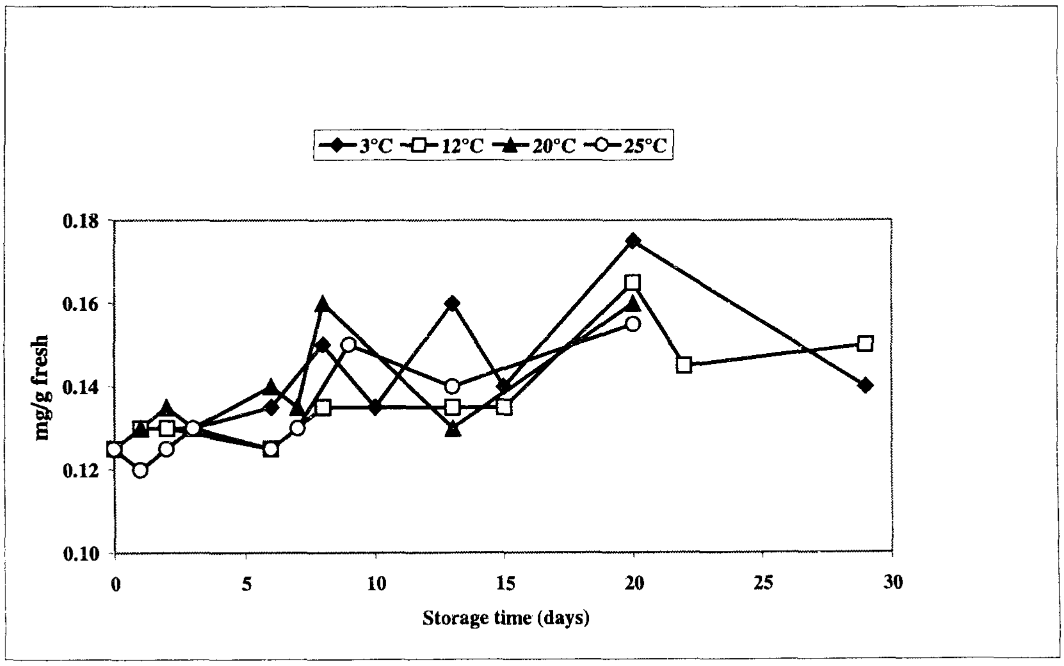


Figure 3.18B: *The amount of ascorbic acid of "RIPE" tomatoes stored at four different temperatures*

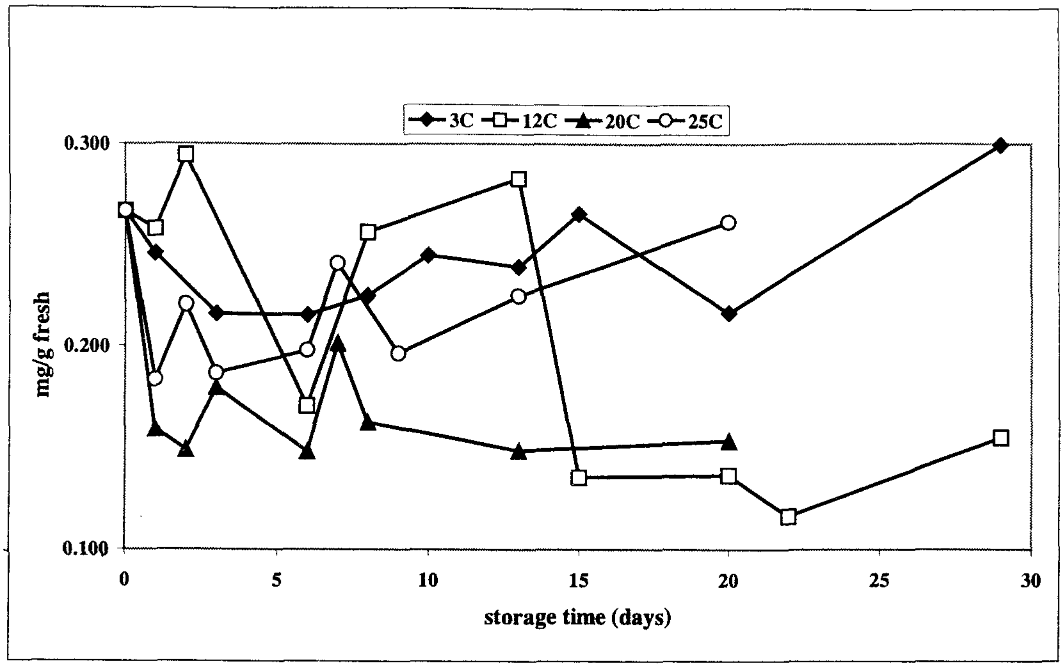


Figure 3.19A: *The amount of pyroglutamic acid of "UNRIPE" tomatoes stored at four different temperatures*

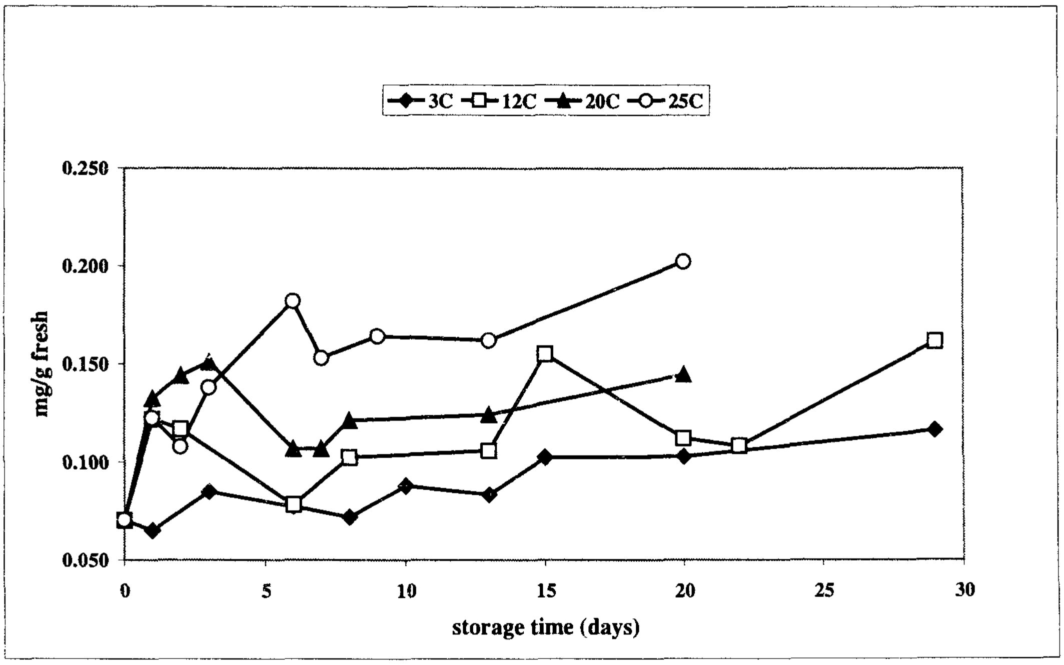


Figure 3.19B: *The amount of pyroglutamic acid of "RIPE" tomatoes stored at four different temperatures*

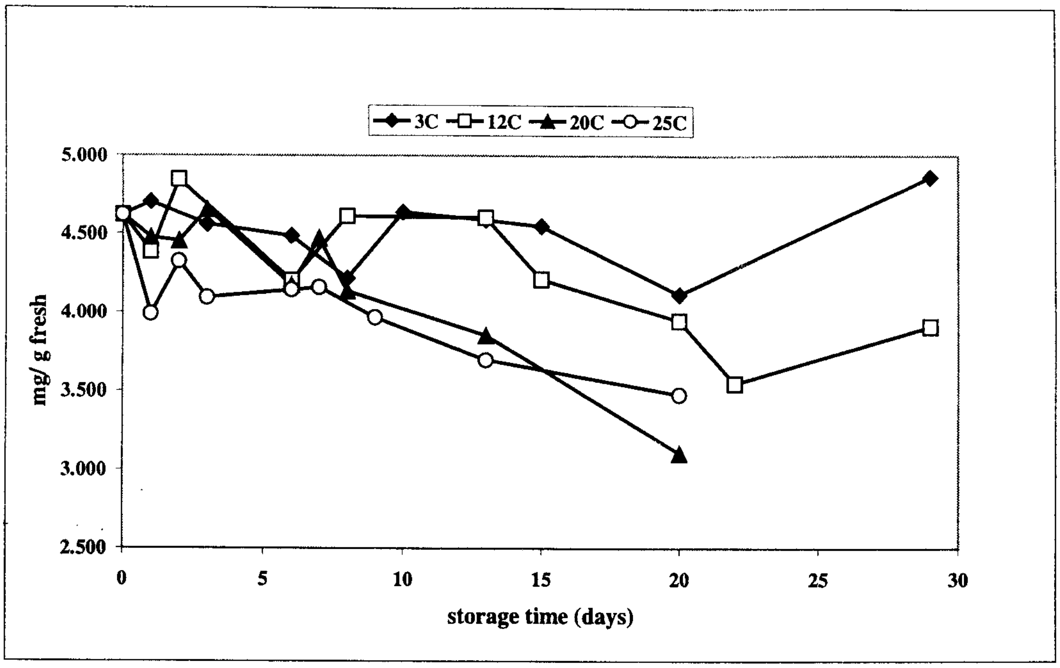


Figure 3.20A: *The amount of citric acid of "UNRIPE" tomatoes stored at fourdifferent temperatures*

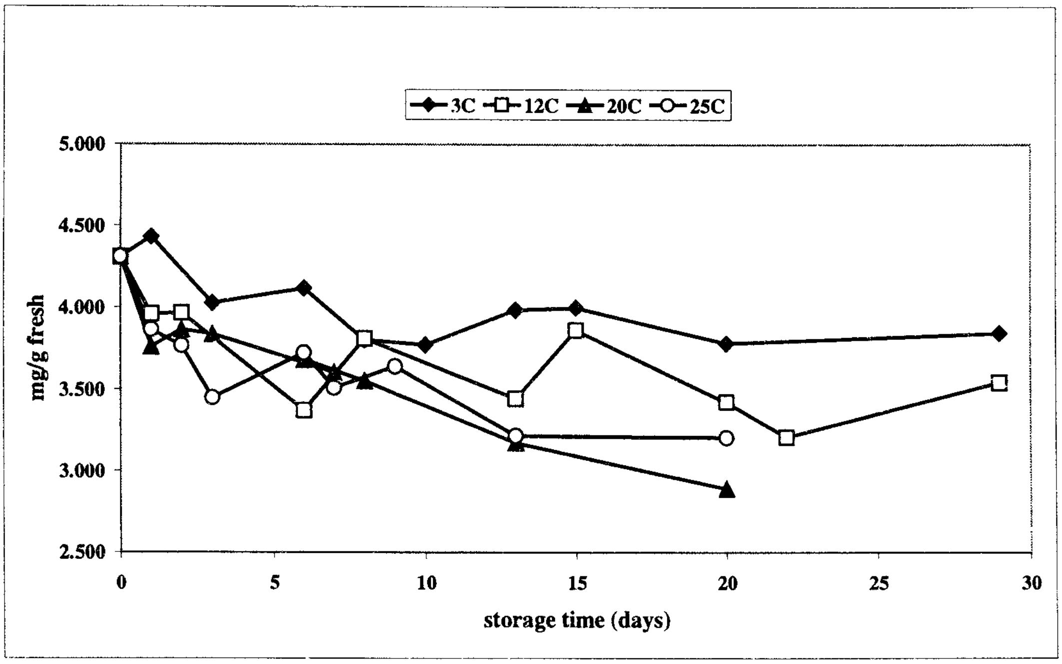


Figure 3.20B: *The amount of citric acid of "RIPE" tomatoes stored at four different temperatures*

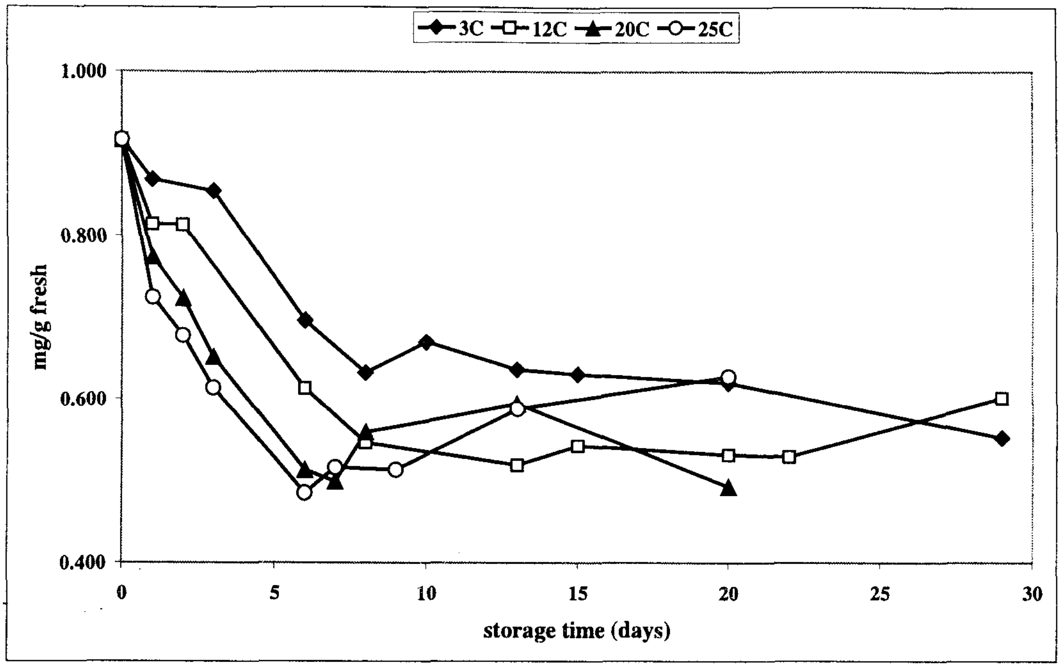


Figure 3.21A: *The amount of malic acid of "UNRIPE" tomatoes stored at four different temperatures*

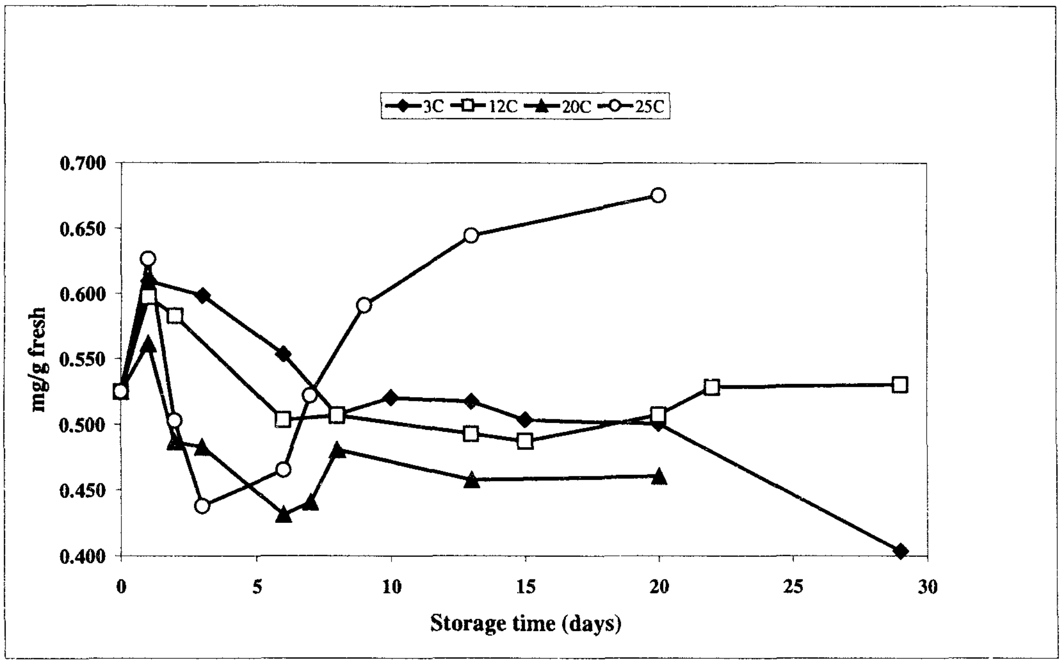


Figure 3.21B: *The amount of malic acid of "RIPE" tomatoes stored at four different temperatures*

3.4.3 Enzymes

Given the very low concentration of enzymes, it is not surprising that no relation could be established between the measured enzyme activity of PME and β-galactosidase and the NIR spectra. However, one exception was observed, namely for PG. For the following discussion it has to be realised that the results described below are not related to the amount of enzyme present in

the tomato, but are related to the total exerted effect of the enzyme on the plant tissue; the amount of PG is far below the NIR detection limit.
The results of a PLS1 analyses of the NIR data against the observed PG activity are given in Table 3.15. A PLS2 analysis, where both the data for PG and for the "slope" are analysed simultaneously, is also given in this Table. The results presented in this Table show that NIR is apparently capable to "measure" the total exerted PG activity.
The results of the PLS2 analysis indicate a correlation, probably a relation between the exerted PG activity and observed "slope". A similar relation could be observed between the PG activity and "distance".

Table 3.15: *Results of a statistical analysis of the NIR prediction of the measured PG activity (PLS1) and NIR prediction of PG activity and "slope" together (PLS2) of all tomato samples (Ripe and Unripe) stored at four different temperatures. Total number of different samples is 70.)*

Statistical information	**Variable**					
	PG		*PG*		*Slope*	
	Data analysis technique					
	PLS1		PL2			
	Calibration	Validation	Calibration	Validation	Calibration	Validation
R	0.964	0.908	0.956	0.894	0.931	0.864
RMSEP	27.8	44.0	30.5	46.7	$4.36\ 10^{-1}$	$6.46\ 10^{-1}$
Bias	$7.63\ 10^{-4}$	$7.35\ 10^{-1}$	$1.95\ 10^{-3}$	$6.41\ 10^{-1}$	$1.86\ 10^{-5}$	$1.31\ 10^{-3}$
Number of outliers	4		4			
Number of PC's	8		8			

3.4.4 *Conclusions on NIR spectroscopy*

- NIR models were generated which accurately predict the inversely related textural properties "slope" and "distance" and to a lesser extend the "water loss". These models included all the tomato samples, irrespective of maturity stage and storage conditions.
- NIR models were generated which rather accurately predict the glucose content as well as the sum of "glucose + fructose". These models included all the tomato samples, irrespective of maturity stage and storage conditions. The prediction model for fructose alone was not too reliable.
- NIR models were generated which rather accurately predict the citric acid and the malic acid content of tomatoes. These models included all the tomato samples, irrespective of maturity stage and storage conditions. No reliable NIR models could be generated for oxalic acid, pyroglutaminic acid and ascorbic acid. The obvious reason for this is, that the amount of these latter acids is too low
- A NIR model was generated which rather accurately predict the PG activity of tomatoes irrespective of maturity stage and storage conditions. Obviously the exerted PG activity, which is the depolymerisation of pectin polymers, is observed rather than the amount of enzyme itself. The result of a PLS2 analysis suggest a relation between the measured PG activity and both the measured "slope" and "distance".

4 RESULTS ON APPLES

4.1 Results on destructive analysis

4.1.1 Physico-chemical characterisation

In Figure 4.1 the dry matter content of "fresh", "midpoint" and "mealy" apples of the varieties Cox, Jonagold and Starking is shown for season 1997.

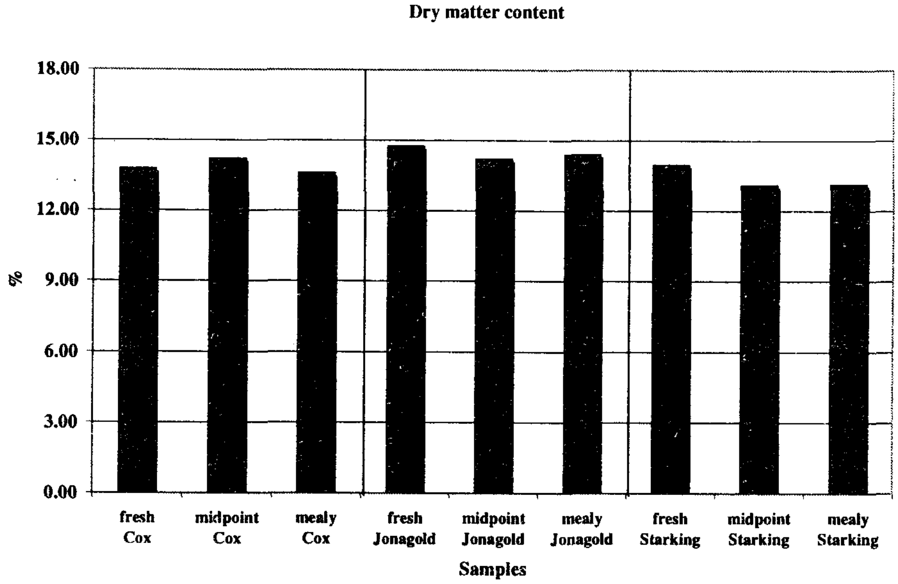


Figure 4.1: *Dry matter content of apples at several degrees of mealiness of season 1997*

The dry matter content of Cox and Jonagold is around 14%, the dry matter content of Starking is around 13%.

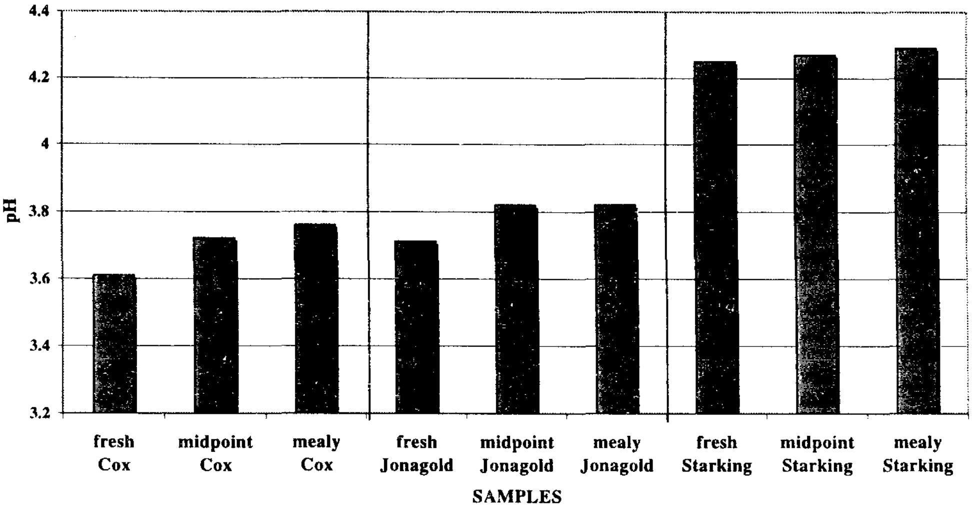


Figure 4.2: *The pH of Cox, Jonagold, and Starking apples at several degrees of mealiness of season 1997*

In Figure 4.2 the pH of "fresh", "midpoint" and "mealy" apples of the varieties Cox, Jonagold and Starking is shown for season 1997. Irrespective of the degree of mealiness the measured pH values of Cox, Jonagold and Starking are respectively 3.7, 3.8 and 4.3.

4.1.2 Analysis of non-volatile taste components

4.1.2.1 Sugars

The sugar content of Cox, Jonagold and Starking apples (season 1997) at several stages of mealiness is shown in Figure 4.3.

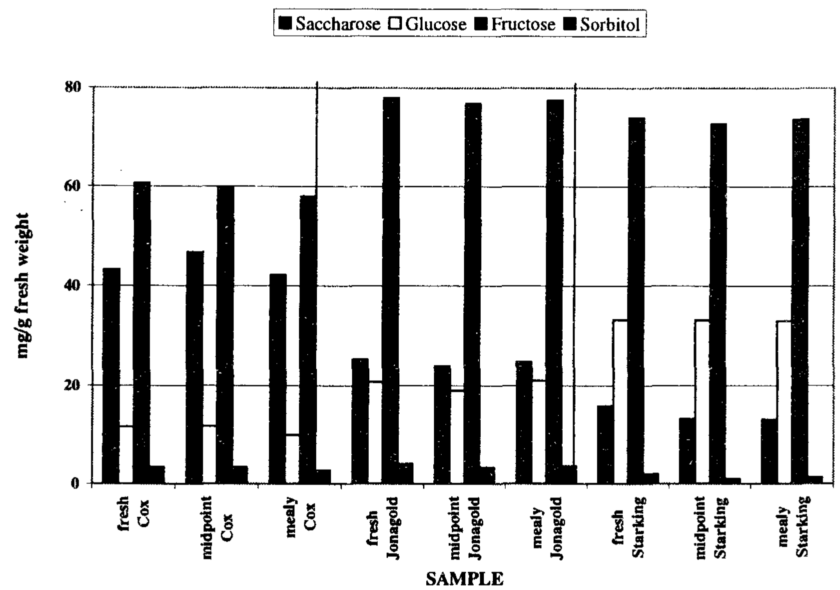


Figure 4.3.: *Sugar content of Cox, Jonagold, and Starking apples at several degress of mealiness*

The total sugar content shown in Figures 4.3 is dependent on variety. In Table 4.1 an overview is given of the percentage of sugars for the varieties studied.

Table 4.1: *Sugar content and calculated sweetness of apple varieties at different levels of mealiness*

Variety	Stage	Sugar % (g/100 g fresh)				Sweetness factor
		Sucrose	*Glucose*	*Fructose*	*Sorbitol*	
Cox	Fresh	4.3	1.2	6.1	0.3	1590
	Mealy	4.2	1.0	5.8	0.3	1520
Jonagold	Fresh	2.5	2.1	7.8	0.4	1790
	Mealy	2.5	2.1	7.8	0.4	1770
Starking	Fresh	1.6	3.3	7.4	0.2	1700
	Mealy	1.6	3.3	7.4	0.1	1670

This Table also includes a "sweetness factor", which is calculated on basis of the sum of the individual amounts of sugars times their individual "sweetness factor". This factor is 100, 74, 174 and 54 respectively for sucrose, glucose, fructose and sorbitol.
From Table 4.1 it can be concluded that:

- The calculated "sweetness factor" is the same for fresh compared to mealy apples
- The "sweetness factor"is independent of the apple variety, despite the different amounts of the individual sugars.

4.1.2.2 Organic acids

Malic acid is the major organic acid in apples. It's concentration is about 100 times that of the second important acid, citric acid. The organic acid content of Cox and Jonagold apples at several stages of mealiness is shown in Figure 4.

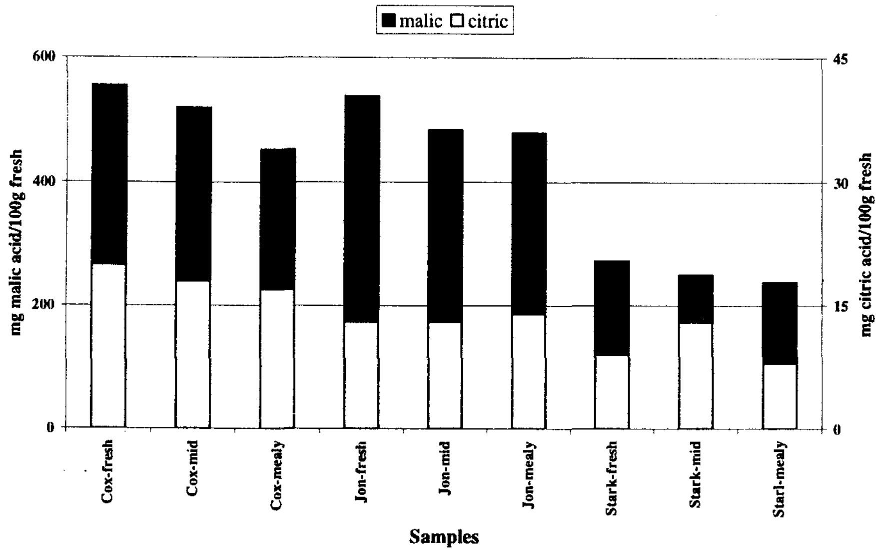
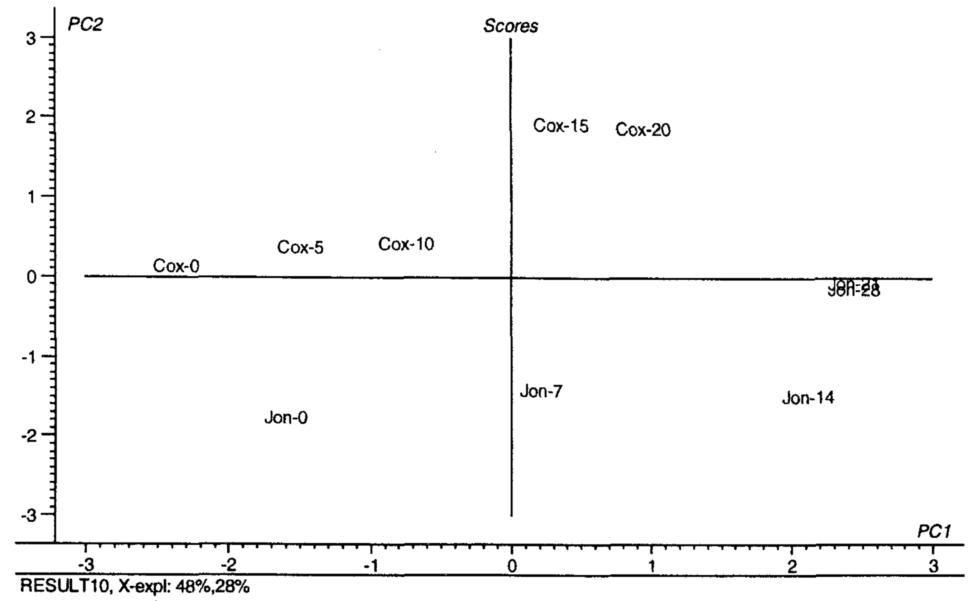


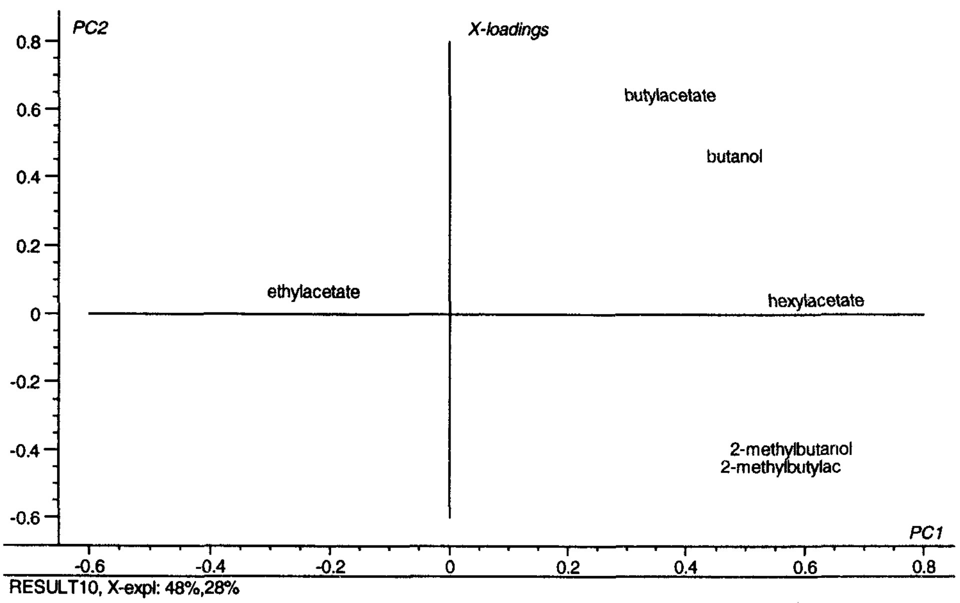
Figure 4.4: *Organic acid content of apples of season 1997*

In contrast to the trend observed for the apples of the 1996 season, where at increasing mealiness the malic acid content decreased, this trend is less pronounced for the apples of season 1997. The malic acid content of Cox, Jonagold and Starking apples is rather independent of the degree in mealiness. The amount of this acid in Starking apples is about half of the value measured in Cox and Jonagold. No clear pattern can be observed for citric acid upon storage/mealiness development. The amount of this acid ranges between 10-20 mg/100g fresh weight.

4.1.3 Additional data analyses of volatile taste components of apples (Cox and Jonagold) of the 1996 season

A PCA analysis was performed for the volatile flavour components of Cox and Jonagold apples simultaneously to see the location of the individual flavour components in relation to the storage of apples an the concomitant mealiness development. Ethanol was omitted from this analysis. Ethanol rather refers to the anaerobic fermentation in relation to the amount of oxygen during storage, than to flavour development upon storage. Furthermore, flavour components, which did not contribute to the loadings plot, neither on PC1, nor on PC2 were also omitted.
The result of this PCA analysis, for the first two PC's is shown in Figures 4.6 A and B. In total six PC's explained 96% of the variance. From this PCA analysis it can be derived that Cox-0 is strongly related with component 3 (ethylacetate), Cox-15 and Cox-20 with component 10 (butylacetate) and Jon-14 with component 13 (2-methylbutylacetate). strongly related with component 3 (ethylacetate), Cox-15 and Cox-20 with component 10 (butylacetate) and Jon-14 with component 13 (2-methylbutylacetate).





Figures. 4.5A,B: *PCA for Cox and Jonagold apples, for flavour components. The distribution of the products (scores: Fig 4.5A) and of the flavour components (loadings: Figure 4.5B) on the first and second PC is shown.*

4.1.4 Additional simultaneous data analyses of volatile and non-volatile taste components with the sensory data of apples (Cox and Jonagold) of the 1996 season

In addition to the sensory analysis both apple varieties were characterised by their sugar, organic acid and flavour content. For this reason a Partial Least Square (PLS) analysis was performed to relate the chemical information with the sensory determined attributes. In this analysis the chemical information contained the sugars (glucose, fructose, sucrose), the most abundant organic acid, malic acid and the flavour components:

- Ethylacetate; mainly present in Jonagold
- butylacetate; present in Cox and Jonagold
- 2-methylbutylacetate; present in Cox and Jonagold
- butanol: present in Cox and Jonagold

- 2-methylbutanol; present in Cox and Jonagold
- hexylacetate; present in Cox and Jonagold

which were shown to be of most important to the apple varieties (see results of PCA analysis in chapter 4.1.3, Figure 4.5). The result of this PLS analysis is given in Table 4.2.

For this analysis three models were used;

- the model containing the chemical information of the Sugars, Acid and Flavour (Model; SAF),
- the model only containing the Flavour information (Model; F),
- the model containing the chemical information on Sugars and Acid (Model; SA).

From the results of this analysis it is obvious that:

- for the texture attributes Firm, Moist and Crispy, relating to fresh apples, the SAF and de F model behave equally well; for the SA model the validation is in all cases worse compared with the SAF and F models,
- for the texture attributes Mealy, Dry and Grainy, relating to mealy apple, the SAF and the F model also behave equally well; for the SA model the validation is in all cases worse compared with the SAF and F models,
- the models for the taste attributes Sweet, Sour and Total aroma are in all cases worse that the models for the texture attributes,
- for the taste attribute Sweet the SAF and the SA models were reasonable, while the validation was unaccepTable low for the F model,
- for the taste attribute Sour performed about equally,
- for the taste attribute Total Aroma the SAF and the SA models were reasonable; no reasonable model could be developed for SA.

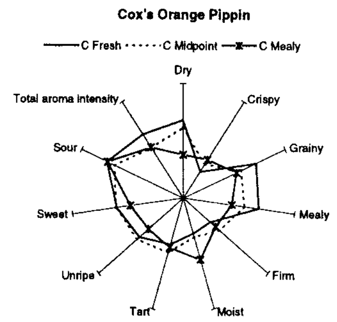
In conclusion it can be said that based on the values of the calibration and validation correlations reliable PLS1 models can be developed able to predict the sensory attributes, base upon the chemical information. Firstly, the PLS1 models are more reliable for texture than for taste attributes and secondly flavour components seem to result in slightly better PLS1 models than sugars and acid.

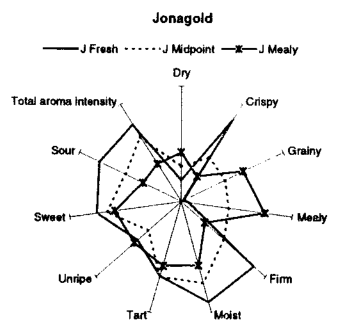
Table 4.2 *Statistical analysis of the most important sugars, organic acids and flavour components with sensory attributes*

Sensory Attribute	Model	Statistical Information					
		Calibration		*Validation*		*Number of PC's*	*Number of outliers*
		R	RMSEP	R	RMSEP		
Fresh apple attributes							
Firm	SAF	0.939	$2.18\ 10^{-1}$	0.858	$3.51\ 10^{-1}$	2	1 (J-21)
	F	0.968	$1.59\ 10^{-1}$	0.898	$3.02\ 10^{-1}$	3	1 (J-21)
	SA	0.942	$2.21\ 10^{-1}$	0.723	$4.73\ 10^{-1}$	2	1 (J-21)
Moist	SAF	0.984	$7.75\ 10^{-2}$	0.947	$1.45\ 10^{-2}$	3	1 (J-28)
	F	0.984	$8.56\ 10^{-2}$	0.845	$2.85\ 10^{-1}$	4	1 (J-07)
	SA	0.984	$9.00\ 10^{-2}$	0.906	$2.21\ 10^{-1}$	3	1 (J-21)
Crispy	SAF	0.976	$1.23\ 10^{-1}$	0.879	$2.88\ 10^{-1}$	2	1 (J-21)
	F	0.979	$1.26\ 10^{-1}$	0.956	$1.92\ 10^{-2}$	3	1 (J-21)
	SA	0.981	$1.25\ 10^{-1}$	0.785	$4.24\ 10^{-1}$	3	1 (J-21)
Mealy apple attributes							
Mealy	SAF	0.984	$7.75\ 10^{-2}$	0.947	$1.45\ 10^{-1}$	3	1 (J-28)
	F	0.981	$1.27\ 10^{-1}$	0.846	$4.19\ 10^{-1}$	4	1 (J-21)
	SA	0.960	$1.96\ 10^{-1}$	0.821	$4.12\ 10^{-1}$	2	1 (J-21)
Dry	SAF	0.981	$7.52\ 10^{-2}$	0.926	$1.81\ 10^{-1}$	4	1 (J-28)
	F	0.957	$1.17\ 10^{-1}$	0.877	$2.00\ 10^{-1}$	3	0
	SA	0.968	$1.25\ 10^{-1}$	0.858	$2.74\ 10^{-1}$	3	1 (J-21)
Grainy	SAF	0.968	$1.31\ 10^{-1}$	0.926	$2.29\ 10^{-1}$	4	1 (J-28)
	F	0.991	$7.75\ 10^{-2}$	0.925	$2.74\ 10^{-1}$	4	1 (J-07)
	SA	0.945	$2.04\ 10^{-1}$	0.821	$3.67\ 10^{-1}$	2	1 (J-21)
Taste Attributes							
Sweet	SAF	0.937	$1.15\ 10^{-1}$	0.794	$2.14\ 10^{-1}$	1	1 (J-28)
	F	0.883	$1.39\ 10^{-1}$	0.599	$2.46\ 10^{-1}$	3	0
	SA	0.957	$9.06\ 10^{-2}$	0.703	$2.30\ 10^{-1}$	3	1 (J-28)
Sour	SAF	0.942	$1.76\ 10^{-1}$	0.787	$3.33\ 10^{-1}$	3	1 (C-0)
	F	0.957	$1.87\ 10^{-1}$	0.847	$3.49\ 10^{-1}$	3	0
	SA	0.936	$2.14\ 10^{-1}$	0.856	$3.17\ 10^{-1}$	2	1 (J-0)
Total aroma	SAF	0.940	$1.18\ 10^{-1}$	0.709	$2.55\ 10^{-1}$	2	1 (C-0)
	F	0.940	$1.18\ 10^{-1}$	0.709	$2.55\ 10^{-1}$	4	1 (C-0)

4.1.5 Results of sensory analysis of season 1997

During the 1997 season emphasis was put on the apple varieties Cox, Starking and Jonagold. Three samples of Cox, Starking and Jonagold apples, characterised by a different degree of mealiness, have been evaluated by the analytical sensory panel. After calculating the standardised scores (z-scores) the data were analysed. In Figure 4.10 the spiderweb diagrams are shown for Cox (Figure 4.6A), Jonagold (Figure 4.6B) and Starking (Figure 4.6C) apples.





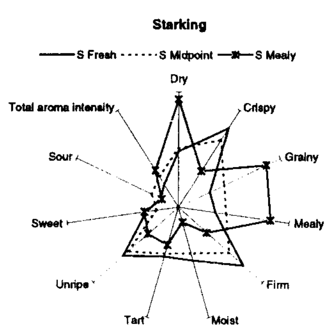
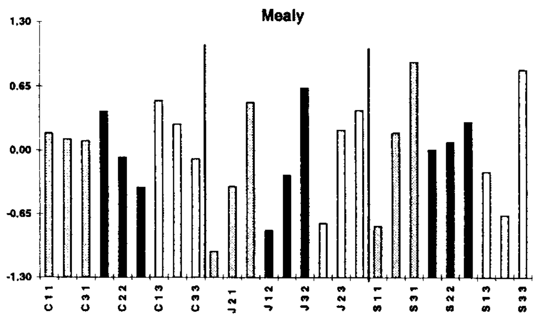
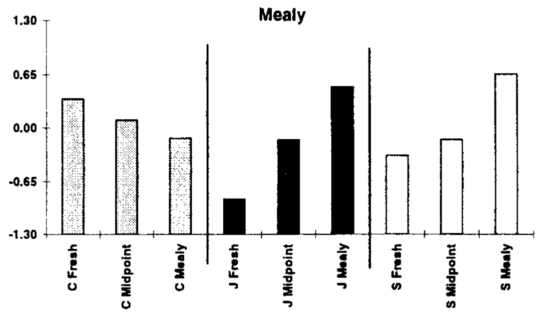
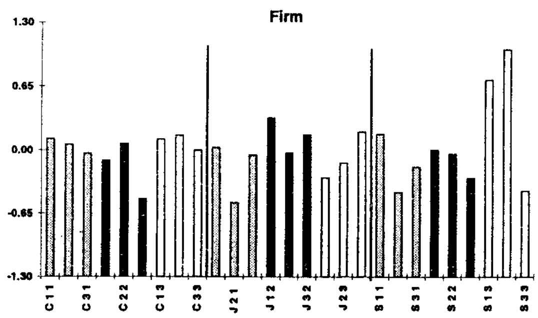


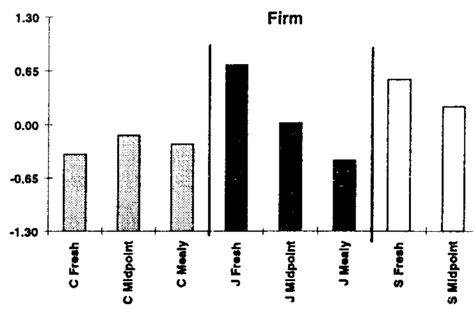
Figure 4.6: *Spiderweb diagrams of the averaged z-scores for all assessors for Cox, Starking and Jonagold apples at three mealiness stages, for three individual sessions and for the average of the three sessions.*

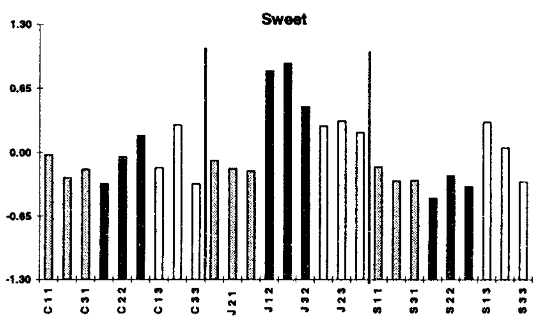
From this Figure it is obvious that both Jonagold and Starking apples become, as expected, more mealy and less firm at increasing degree of mealiness. However, the Cox apples showed an unexpected behaviour. Firstly, the "mealy" apples are, based on the sensory measurements, less mealy than the "fresh" apples. Secondly, in contrast to the previous observations the development of mealiness of the Cox apples is substantially less pronounced as compared to the previous season (season 1996). The expected behaviour for Jonagold and Starking apples, and the anomalous behaviour of Cox apples is presented in the averaged z-scores of several individual sensory attributes.

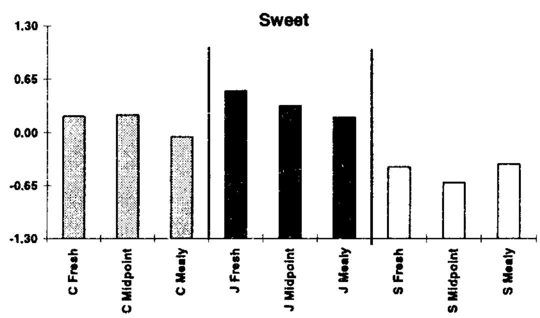

Mealy
1.30
0.65
0.00
-0.65
-1.30
C11
C31
C22
C13
C33
J21
J12
J32
J23
S11
S31
S22
S13
S33

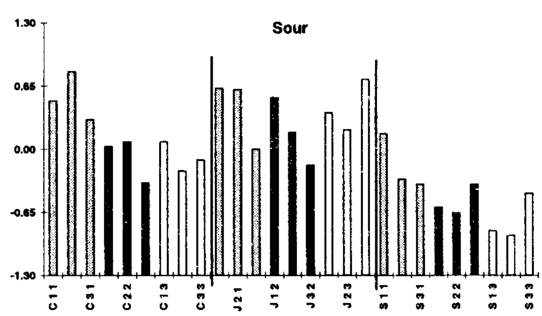

Mealy
1.30
0.65
0.00
-0.65
-1.30
C Fresh
C Midpoint
C Mealy
J Fresh
J Midpoint
J Mealy
S Fresh
S Midpoint
S Mealy

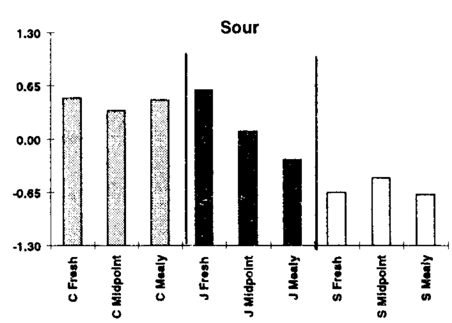

Firm
1.30
0.65
0.00
-0.65
-1.30
C11
C31
C22
C13
C33
J21
J12
J32
J23
S11
S31
S22
S13
S33


Firm
1.30
0.65
0.00
-0.65
-1.30
C Fresh
C Midpoint
C Mealy
J Fresh
J Midpoint
J Mealy
S Fresh
S Midpoint


Sweet
1.30
0.65
0.00
-0.65
-1.30
C11
C31
C22
C13
C33
J21
J12
J32
J23
S11
S31
S22
S13
S33


Sweet
1.30
0.65
0.00
-0.65
-1.30
C Fresh
C Midpoint
C Mealy
J Fresh
J Midpoint
J Mealy
S Fresh
S Midpoint
S Mealy


Sour
1.30
0.65
0.00
-0.65
-1.30
C11
C31
C22
C13
C33
J21
J12
J32
J23
S11
S31
S22
S13
S33


Sour
1.30
0.65
0.00
-0.65
-1.30
C Fresh
C Midpoint
C Mealy
J Fresh
J Midpoint
J Mealy
S Fresh
S Midpoint
S Mealy

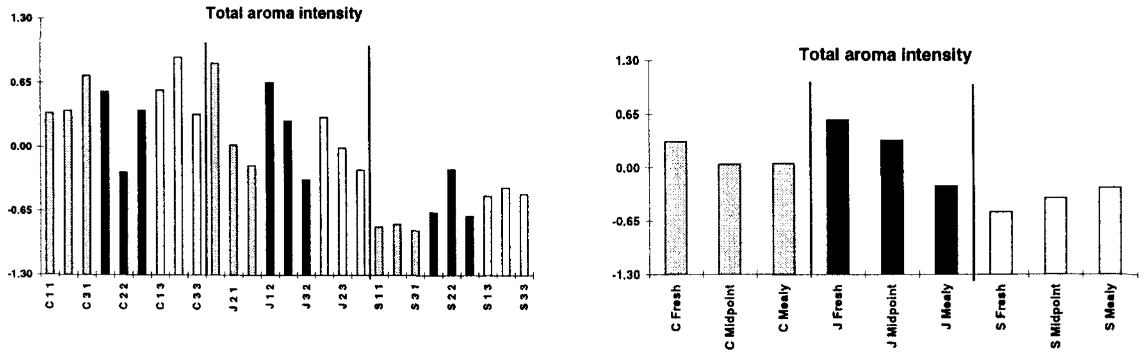
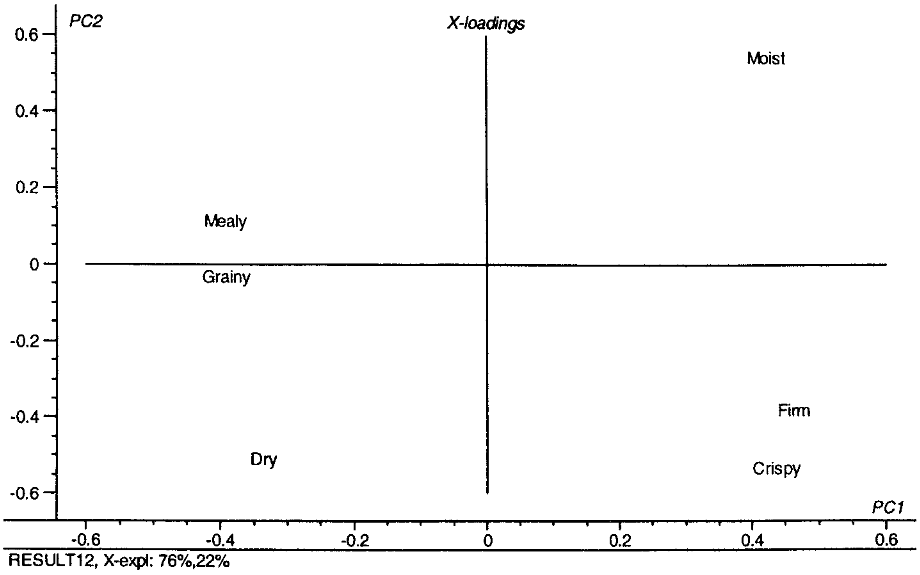


Figure 4.7 *Averaged z-scores of the attributes "mealy", "firm", "sweet", "sour" and "total aroma intensity" for Cox, Jonagold, and Starking apples, at three mealiness stages, for three individual sessions and for the average of the three sessions.*

From this Figure it can be seen that a large variance can be observed between the z-scores of a given attribute for the three sensory sessions. **The obvious reason for this is the large variance of the individual apples within one batch.** Despite this large variance some general conclusions can be drawn relating the sensory information presented in Figures 4.11 and the chemical information given in Table 4.1 on basis of PCA analysis.

A PCA analysis for the mouthfeel attributes of both Jonagold and Starking apples is given in Figure 4.12 For Jonagold and Starking apples the range from fresh to mealy is obvious. Fresh apples are firm, crispy and moist and mealy apples are grainy, dry and mealy. For the different samples of Cox's apples is no range from fresh to mealy present. The Cox apples are omitted from the analysis given their non-discriminate sensory behaviour.

The PCA-plot for the non-volatile taste components and taste attributes (Figure 4.13) shows that there are differences in between varieties, but not so much within varieties. On the first PC the attribute sour and the malic acid content together with the proton concentration are grouped together. No relation can be observed for the attribute sweetness with neither the amount of glucose, fructose and the sweetness factor.



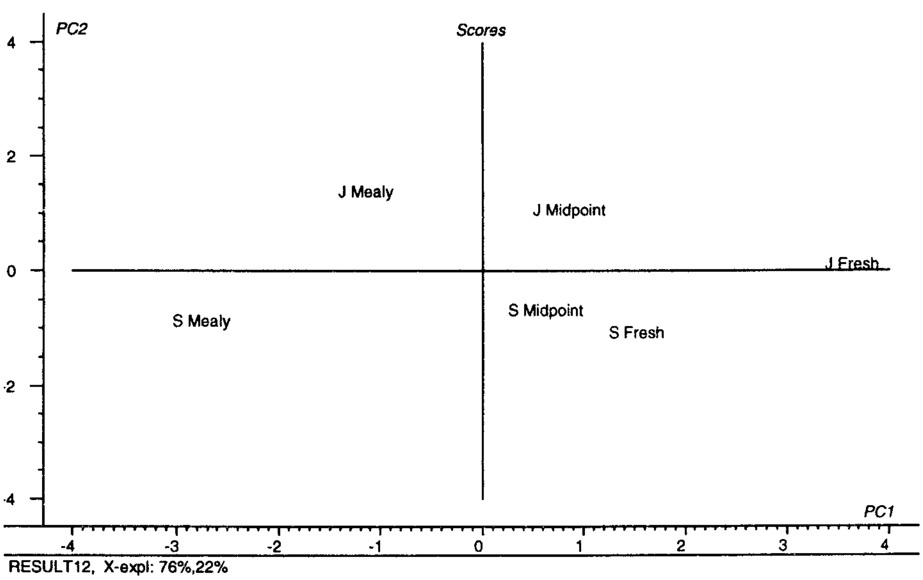


Figure. 4.8 *Scores-, and loadings plot of PCA analysis of Jonagold and Starking apples at several degrees of mealiness for mouthfeel attributes*

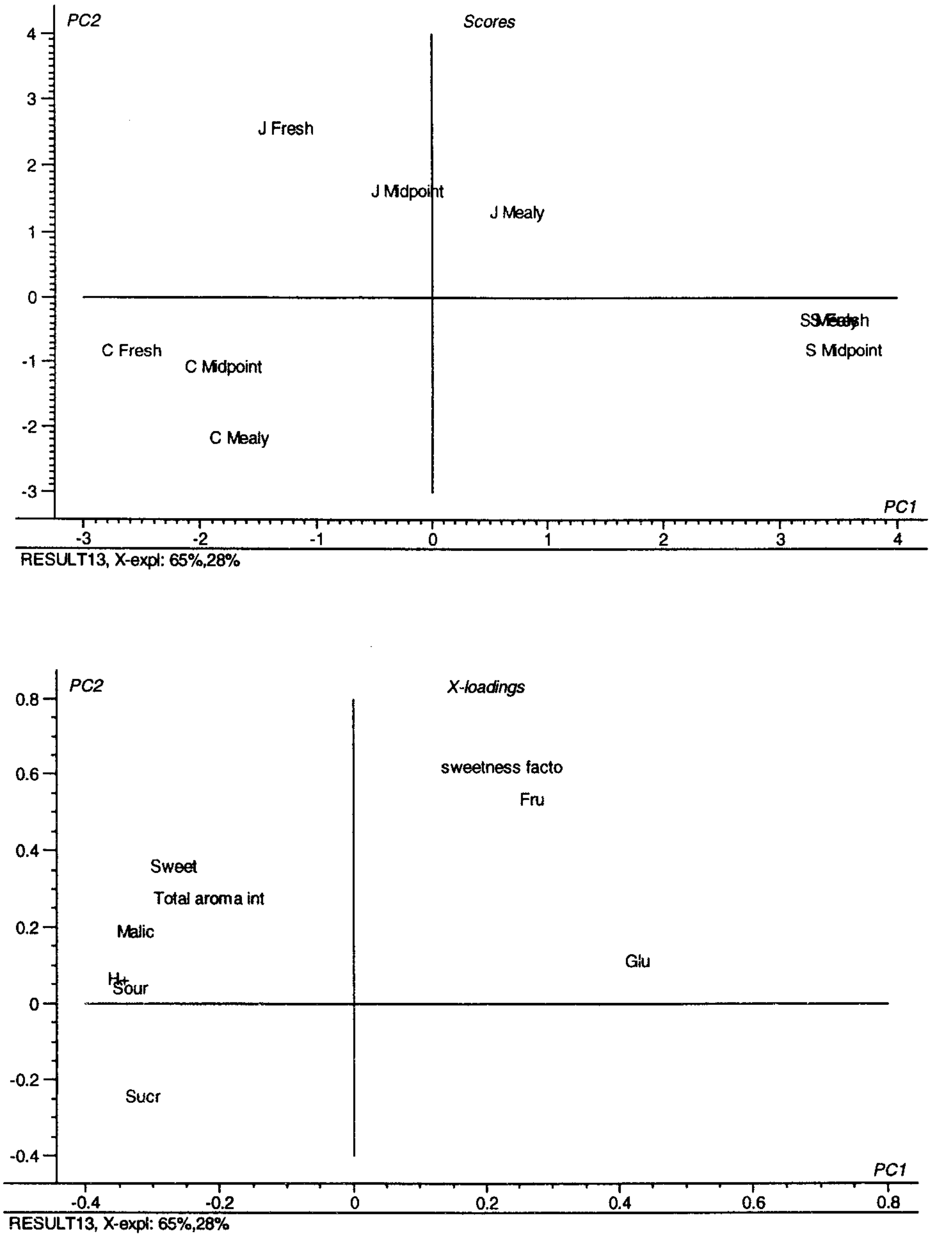


Figure 4.9 *Scores-, and loadings plot of PCA analysis of Jonagold, Starking and Cox apples at several degrees of mealiness for taste attributes and chemically determined non-volatile taste components.*

In order to get an idea about the actual distances between the mean values for the respective attributes of the three stages of a variety the data are checked with the help of ANOVA. In Table 4.4 the significant differences between the apples are shown. Only the significant differences within one variety are shown. The different letters from low (a) to high (b,c) show the significant differences on the 95% confidence limits ($p \leq 0.05$). Within each column and variety different letters indicate samples which are significantly different.

Table 4.4 *Significant differences between fresh, midpoint and mealy apples for Cox's Orange Pippin, Jonagold and Starking apples for the different attributes.*

		Crispy	dry	firm	Grainy	Mealy	moist	sour	sweet	tart	aroma	unripe
Cox	Fresh	a	a	a	a	a	a	a	a	a	a	a
	Midpoint	a	a	a	a	a	a	a	a	a	a	a
	Mealy	a	a	a	a	a	a	a	a	a	a	a
Jonagold	Fresh	b	a	a	a	a	b	a	a	b	b	b
	Midpoint	a	a	a	b	b	a	a	a	ab	ab	ab
	Mealy	a	a	a	b	c	a	a	a	a	a	a
Starking	Fresh	b	a	b	a	a	a	a	a	a	a	a
	Midpoint	a	a	ab	a	a	a	a	a	a	a	a
	Mealy	a	b	a	b	b	a	a	a	a	a	a

There are no significant differences between the apple samples for any attribute of Cox's Orange Pippin. For Jonagold apples there are significant difference for the attributes crispy, grainy, mealy, moist, tart, aroma and unripe/green aroma. For Starking apples there are significant differences for the attributes crispy, dry, firm, grainy and mealy.
The most important issue for was to create different levels of mealiness stages within one variety. The varieties Jonagold and Starking met the conceptual ideas, but Cox's apples were obviously already to far in the process of tissue breakdown when the experiment started.

In Table 4.2 a statistical analysis is presented, relating the major chemical components analyzed (sugars, acid, volatiles) with sensory attributes of the 1996 data. However, for the 1997 data no statistical relevant relations could be established between the chemical components analyzed (sugars, acid) and the sensory attributes.
The obvious reason for this is probably the large variance in the data, both the sensory as well as the chemical data. This variance is probably caused by the large variance of the apples within one batch.

4.1.6 Biological variance between batches

Based on the sensory results of 1997 a large variance between was observed (see Figure 4.11). In order to address this problem of variances between batches of both Cox and Elstar apples were sensorically analyzed. The apples differed in:

- picking time; early and late,
- size; small and large
- storage conditions; low (80%) and high (90%) relative humidity
- storage time.

In Figure 4.10 a bar-diagram for Elstar apples, for all the experimental variables and all the measuring points for the attribute mealy is shown. The black bars represent the apples of the first measuring point; these apples were not stored. The white bars represent the apples stored at a high relative humidity and the gray bars represent the apples stored at a low relative humidity. This bar-diagram of the most important attribute mealiness shows that there is no trend in these data. All the other attributes do give the same erratic results (data not shown). Similar (erratic) results were obtained for Cox apples (data not shown). The apples do not meet the expectation that at least some of the variables do have an effect on the perceived attributes. For this reason no further attention was paid to the analysis of these data.

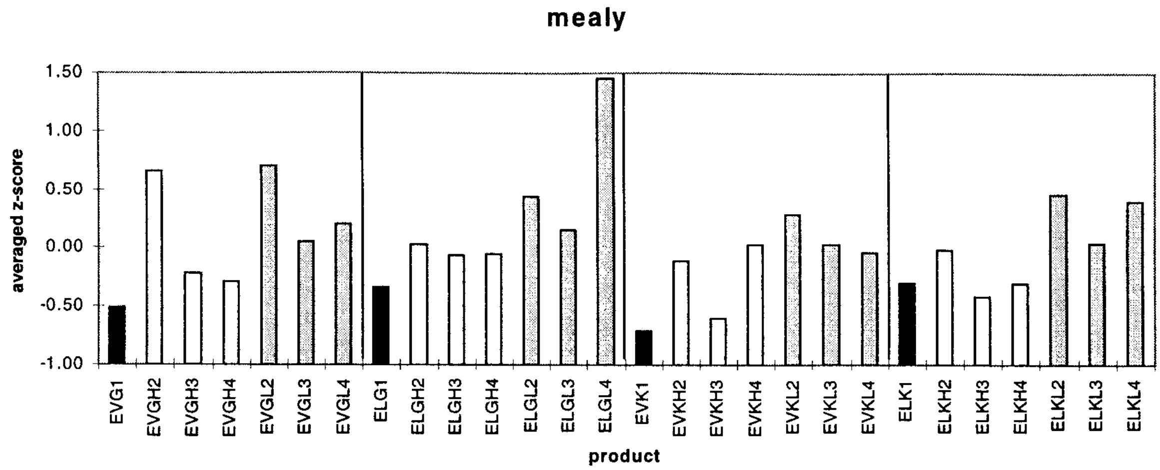


Figure 4.14 *Bar-diagram of Elstar (E) apples for the attribute mealy for all the experimental conditions for four measuring points during storage. Picking time early (V) and late (L), size small (K) and large (G), relative humidity low (L) and high (H) and four measuring points (1 to 4). Example: EVGH2 → Elstar, early picked, big size, high humidity, measuring point 2.*

It is assumed that the following factors are causing the above described erratic results:

- Origin of the apples: it's not sure that all the apples originate from one grower because the apples come from different auctions.
- Picking time: the apples were not picked with one week in between; the biological maturity of the apples was not taken into consideration..
- Size: the range of sizes (small and big) overlapped each other.
- Relative humidity: the apples were supposed to be stored at 80% and 90% humidity. The conditions for 90% humidity were not controlled, so the storage conditions for this humidity are very uncertain.

4.1.7 Non-destructive analysis: NIR

Apples of variety Elstar, at four mealiness levels, were first measured by NIR spectroscopy and then subjected to chemical and sensory analysis (**expert panel**). Every apple was treated as a separate sample. Partial Least Squares analysis (PLS) was used to develop models for predicting sensorial perceived mealiness and malic acid content based on spectral data. Malic acid was considered an important chemical parameter indicative for mealiness, since there is a high negative correlation between malic acid content and mealiness of the fruit (data not showed).
In table 4.6 the statistical results for mealiness scores and malic acid content are given.

Table 4.6 *Results of a statistical analysis of the NIR prediction of the sensory perceived "mealiness" and chemically determined "malic acid" content of individually analysed apples (Elstar).*

Statistical information	**Variable**			
	Mealiness		*Malic acid*	
	Calibration	Validation	Calibration	Validation
R	0.96	0.80	0.95	0.81
RMSEP	0.35	0.72	29	57
Number of PC's	2		2	

Also in this case it is seen that the Rval for both the sensory mealiness as for the malic acid content is relatively low. However, taking the relatively small data set into account, the results of the measurements on individual apples (using an expert panel) indicate a higher value for both Rcal as well as Rval. compared with the values obtained for batches of apples (using an analytical sensory panel).

In conclusion

It can be argued that working with **batches** in combination with an **analytical sensory panel** is favoured:

- if the sample variance within a batch is low, proper correlations (if existent) can be observed between sensory measurements and instrument, analytical measurements; for example see the results on apples of 1996, chapter 4.1.4.2
- if the sample variance within a batch is high, no correlations (if existent) can be established between sensory measurements and instrument, analytical measurements; for example see the results on apples of 1997, chapter 4.1.4.3.

It can be argued that working with **individual products (apples)** in combination with an **expert sensory panel** is favoured :

- if the sample variance within a batch is low, their is no advantage in working with an analytical panel, since an analytical panel is o/a. working with a continuous scale and an expert panel with a fixed scale; this latter is going at the expense of the resolution of the sensory data.
- if the sample variance within a batch is high, an expert panel is preferred if both sensory and instrumental, analytical measurements can be performed on the same product.

5 DISCUSSION

5.1 General remarks

One of the main problems encountered in this projects was dealing with the product variance within a batch, especially for apples. Based on the results for apples, the batch variance for the apples studied in 1996 (see chapter 4.1.4.2) was thus, that proper statistical relations between sensory perceived attributes and analytical, instrumental properties could be established. In contrast to the 1996 apples, the batch variance of the apples studied in 1997 (see chapter 4.1.4.3) was that high, that no relations between sensory data, using an analytical sensory panel, and instrumental data could be established. However, performing both instrumental and sensory measurements (by an expert panel) on individual apples reliable relations could be established between instrumental and sensory data.
With regard to the batch variance of tomatoes the following can be concluded. Tomatoes were harvested at two maturity stages; colour stage 5 ("Unripe") and colour stage 7 ("Ripe"). These two sets of tomatoes were stored at four different temperatures, for about four weeks. Based on the results (see chapter 3), both fundamental models as well as statistical models were developed , all characterised by an explained part of about 90%. Therefore it can be concluded, that the batch variance of tomatoes is low and that the selection criterion "colour stage" is therefore a (very) good criterion.

5.2 Tomatoes

Tomatoes were harvested at two maturity stages; colour stage 6 ("Unripe") and colour stage 8 ("Ripe"). Next they were stored at 4 temperatures; 3 ^{0}C (chilling injury temperature), 12 ^{0}C (optimal storage temperature), 20 ^{0}C and 25 ^{0}C , all at a relative humidity of about 90%. During storage samples were withdrawn for further analyses.

Non-destructive analysis were performed on individual tomatoes:

- Compression measurements: values determined were slope (N/m), distance (m) and tomato-diameter (m).
- Near Infra Red (NIR) measurements.

Destructive measurements were performed on the homogenate of 20 tomatoes representing one storage- time interval. The destructive measurements performed comprised the analysis of the:

- dry matter content
- abundant sugars: glucose, fructose, saccharose
- abundant acids: citric acid, fumaric acid, oxalic acid malic acid and pyroglutamic acid
- vitamin c: ascorbic acid and dehydro-ascorbic acid
- protein
- enzymes: pectin methyl esterase, poly-galacturonase, β-galactosidase

Based on these results both fundamental an statistical models were developed.
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 (Tijskens *et al.* 1997), in this case processes relating to the change in firmness of tomatoes.

The **statistical models** are empirical and relate the changes in product properties (e.g. firmness, sugar content) with changes in the Near Infra Red spectrum of intact tomatoes. In using a large sample set reliable (statistical) NIR prediction models can be build.

Fundamental models build in this project are models;
Based on non-destructive measurements:
- a model on Firmness, based on compression measurements
- a model on water loss

Based on destructive measurements:
- a model on the behaviour of PG activity
- a model on the behaviour of PE activity
- a model on the behaviour of β-galactosidase activity

Statistical models build in this project are models :
Based on non-destructive measurements:
- a NIR model predicting the Firmness, based on non-destructive compression measurements
- a NIR model predicting the water loss

Based on destructive measurements:
- NIR models predicting the sugar content (glucose and fructose)
- NIR models predicting the organic acid content (citric and malic acid)
- NIR model predicting the PG activity.
- NIR model with simultaneously predict the PG activity and Firmness

5.3 Apples

The research on apples comprised the following aspects:
- analytical sensory research of apple varieties, each variety characterised by a range in "mealiness" ; from "fresh" to "very-mealy" apples
- destructive analytical instrumental research to characterise the
  - volatile flavour components in relation to the mealiness stage of apples/apple varieties
  - non-volatile flavour components (sugars, organic acids) in relation to mealiness stage of apples/apple varieties
- non-destructive analytical instrumental research based on NIR spectroscopy to statistically relate the sensory perceived mealiness NIR spectra.

For the 1996 apples the batch variance was low. The following statistical models were build:

A Models relating sensory analysis with destructive chemical analysis
Statistical models relating the change in chemical composition (volatiles, sugars and organic acid) of the apples with the sensory attributes. For these models the apple varieties Cox and Jonagold were analysed simultaneously.
- The reliability of these models was greater for the texture than for the taste attributes
- The highest reliability of the models was obtained when the volatiles, sugars and organic acid were used as input information. Similar results in reliability were obtained when either the volatiles or the "sugars + organic acid" were used as input information.
- In all cases malic acid, the major organic acid of apples, highly contributed to these statistical models.

B *NIR models*

- For the apple varieties Jonagold and Cox, NIR models were developed relating the NIR spectra with the sensory attributes "mealiness", "firmness" and "Sour", both for the individual varieties as well as for the varieties together.
- A NIR model was developed relating NIR spectra with the malic acid content, for both varieties(Cox and Jonagold) simultaneously.

For the 1997 apples the batch variance was high. For this reason no statistical models could be developed on basis of information obtained with batches.
In order to avoid the batch variance both sensory (expert panel) and analytical measurements (NIR on intact apples; instrumental to determine the malic acid content) were performed on one apple. Based on this approach reliable NIR models could be build able to predict both the sensory perceived mealiness as well as the malic acid content.

6 Conclusions

6.1 Conclusions on tomatoes

6.1.1 In general

- The color of tomatoes of *cv.* Tradiro are a very suiTable indicator of the maturity stage at harvest, resulting a low batch variance.
- The **mathematical models** developed are capable to predict the changes in *"firmness"*, *"water loss"* and *"enzyme activity* (PG, PE, β-galctosidase)" in time of tomatoes of *cv.* Tradiro given their maturity stage at harvest and storage temperature.
- The **NIR models** are capable to predict the present firmness, water loss, as well as sugar and organic acid content of tomatoes of cv Tradiro, irrespective of their maturity at harvest and storage conditions.

6.1.2 Conclusions on mathematical modelling

- Mathematical models were formulated based on fundamental processes causing the measured changes. The measured changes modeled were respectively related to firmness (slope and distance), water loss, and PG activity.
- The variability accounted for was for all models at least 90%, indicating the reliability of the assumed underlying mechanisms used to build the models.
- The models developed can be used to predict changes in firmness given the maturity stage at harvest and storage temperature

6.1.3 Conclusions on NIR models

- NIR models were generated which accurately predict the inversely related textural properties "slope" and "distance" and to a lesser extend the "water loss"". These models included all the tomato samples, irrespective of maturity stage and storage conditions.
- NIR models were generated which rather accurately predict the glucose content as well as the sum of "glucose + fructose". These models included all the tomato samples, irrespective of maturity stage and storage conditions.
- NIR models were generated which rather accurately predict the citric acid and the malic acid content of tomatoes. These models included all the tomato samples, irrespective of maturity stage and storage conditions. No reliable NIR models could be generated for oxalic acid, pyroglutaminic acid and ascorbic acid. The obvious reason for this is, that the amount of these latter acids is too low
- A NIR model was generated which rather accurately predict the PG activity of tomatoes irrespective of maturity stage and storage conditions. Obviously the exerted PG activity, which is the depolymerisation of pectin polymers, is observed rather than the amount of enzyme itself. The result of a PLS2 analysis suggest a relation between the measured PG activity and both the measured "slope" and "distance".
- The NIR models described above include all the tomato samples, irrespective of maturity stage and storage conditions.

6.2 Conclusions on apples

6.2.1 In general

- The **batch variance** of apples was dependant on the season. In 1996 the batch variance for apples was low, in 1997 the batch variance for apples was high. In the latter case no predictive (batch) models could be build. Crucial for further research is the control of the batch variance.
- For apples a set of sensory attributes was generated which include both texture and taste attributes.

6.2.2 Conclusions on statistical models

- For the 1996 season the NIR models were generated capable to predict sensory perceived texture attributes based on batches. These models included both apple varieties (Cox and Jonagold).
- NIR models were generated which rather accurately predict the malic acid content. These models included both apple varieties (Cox and Jonagold).
- "Chemical" models were generated which rather accurately predict the sensory perceived texture and taste attributes on basis of the change in sugars, malic acid and volatiles. These models included both apple varieties (Cox and Jonagold).
- For the 1996 season a NIR model was generated capable to predict sensory perceived texture attribute "*mealiness*" based on measurements of individual apples. These model was based on the apple variety Elstar.
- For the 1996 season a NIR model was generated capable to predict the malic acid content of Elstar apples.

6.3 General conclusions

- Near Infra Red spectroscopy appears to be a strong tool to predict
  - sensory perceived texture and taste attributes
  - mechanical properties,
  - major chemical constituents contributing to the taste of a product.
- Mathematical models are capable to predict the product behavior with time and temperature
- Knowledge about batch variance is crucial both for mathematical and statisticalmodels.

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