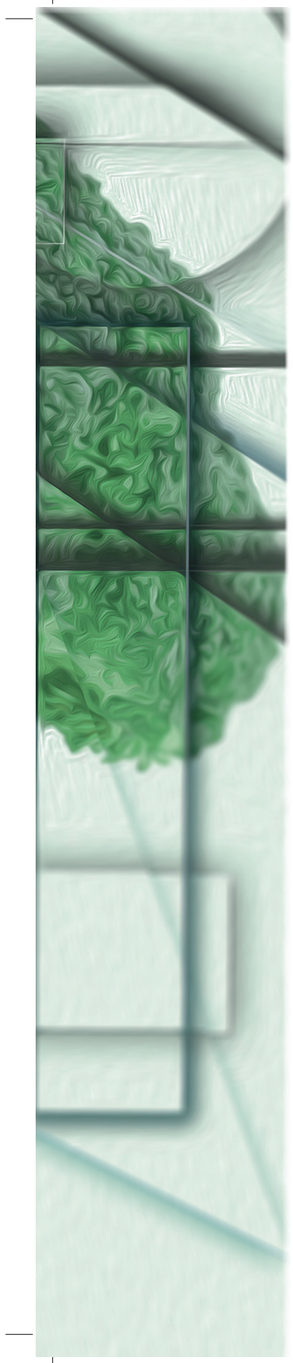


FOOD PROCESSING AND HEALTH: A case on the glucosinolate - myrosinase system in dried broccoli

Teresa Oliviero

FOOD PROCESSING AND HEALTH:
A case on the glucosinolate - myrosinase system in dried broccoli

Teresa Oliviero

INVITATION

You are cordially invited to attend the public defence of the thesis entitled:

FOOD PROCESSING AND HEALTH:
A case on glucosinolate – myrosinase system in dried broccoli

On Tuesday, 17 December 2013
At 11.00 a.m. in the Aula
Of Wageningen University,
Generaal Foulkesweg 1a in
Wageningen.

You are kindly invited to join the reception that will be held in the Aula after the ceremony.

Teresa Oliviero
teresa.oliviero@wur.nl

PARANYMPHS
Elsa Antunes Fernandes
elsa.antunesfernandes@wur.nl
and
Daylan Tzompa Sosa
daylan.tzompasosa@wur.nl

In the past years several studies have been focused on a class of compounds that occurs in *Brassica* vegetables. These compounds, called glucosinolates (GLs), have drawn the attention of many researchers. In fact, some of their breakdown products show anticarcinogenic bioactivity *in vitro* and *in vivo* studies. GLs are hydrolysed by myrosinase (MYR), an endogenous plant enzyme, upon damage of the plant matrix (mastication, mincing etc.). Among the breakdown products isothiocynates (ITCs) show anticarcinogenic bioactivity. Food processing, such as canning, cooking, drying etc., affect the final intake of ITCs. In fact, high temperature inactivates MYR and degrade GLs. Air drying is a widely used technology that can dramatically affect the GLs concentration and MYR activity. The first aim of this study was to kinetically model the effect of temperature and water on GLs degradation and MYR inactivation in broccoli (*Brassica oleracea* var. *italica*). These models were used to optimize air drying conditions to obtain broccoli product with certain GL levels and MYR activity. The second aim was to investigate the *in vivo* absorption of ITCs after consuming broccoli products with different GLs, MYR and ITCs profiles.

During drying broccoli is subjected to changes in water content and temperature which affect the occurrence of GLs and MYR. Hence, the influence of water content and temperature on GLs degradation and MYR inactivation kinetics was studied. Water content was found to have a remarkable effect on GLs and MYR kinetic parameters. The kinetics of GLs degradation, MYR inactivation and Vitamin C were used to optimize air drying temperature trajectories (dynamic optimization) to reduce GLs, MYR and Vitamin C loss upon drying. The results show that the optimized temperature trajectories led to remarkable improvement of GLs, MYR and Vitamin C retention.

Then the effect of broccoli products with different GLs, MYR and ITC profiles on the ITC absorption was investigated *in vivo*. In particular, the role of different MYR activity in the broccoli products on ITC formation, bioavailability and excretion kinetics was investigated. Broccoli batches were differently processed in order to obtain five dried products with different GLs, MYR and ITC profiles. These products were consumed by 15 human volunteers in a cross-over design study. After consumption of these broccoli products, 24 h urine samples were collected and ITCs metabolites were determined. The consumption of broccoli product with 20% residual MYR activity showed similar bioavailability to the broccoli product with intact MYR activity. Moreover, the consumption of broccoli product with 2% residual MYR showed a significant higher bioavailability values than the broccoli product with no residual MYR activity.

This research shows that process optimization is a useful tool to produce dry broccoli in which GLs, Vitamin C and MYR are retained. Moreover, certain levels of GLs and MYR retention can be associated to specific ITCs excretion kinetics and bioavailability, hence with specific health effect.

Food processing and health:

A case on the glucosinolate-myrosinase system in dried broccoli

Teresa Oliviero

Thesis committee

Promotors

Prof. Dr. Martinus A. J. S. van Boekel
Professor of Product Design and Quality Management
Wageningen University

Co-promotors

Dr. Matthijs Dekker
Associate Professor, Food Quality and Design Group
Wageningen University

Dr. Ruud Verkerk
Assistant Professor, Food Quality and Design Group
Wageningen University

Other members

Prof. Dr. Harry J. Wichers, Wageningen University

Prof. Dr. Nicoletta Pellegrini, University of Parma, Italy

Dr. Atze Jan van der Goot, Wageningen University

Dr. Peter C.H. Hollman, Wageningen University

This research was conducted under the auspices of the Graduate schools VLAG (Advanced studies in Food Technology, Agrobiotechnology, Nutrition and Health Sciences).

Food processing and health:

A case on the glucosinolate-myrosinase system in dried broccoli

Teresa Oliviero

Thesis

submitted in fulfilment of the requirements for the degree of doctor
at Wageningen University
by the authority of the Rector Magnificus
Prof. Dr M. J. Kropff,
in the presence of the
Thesis Committee appointed by the Academic Board
on Wednesday 17 December 2013
at 11 a.m. in the Aula.

Teresa Oliviero

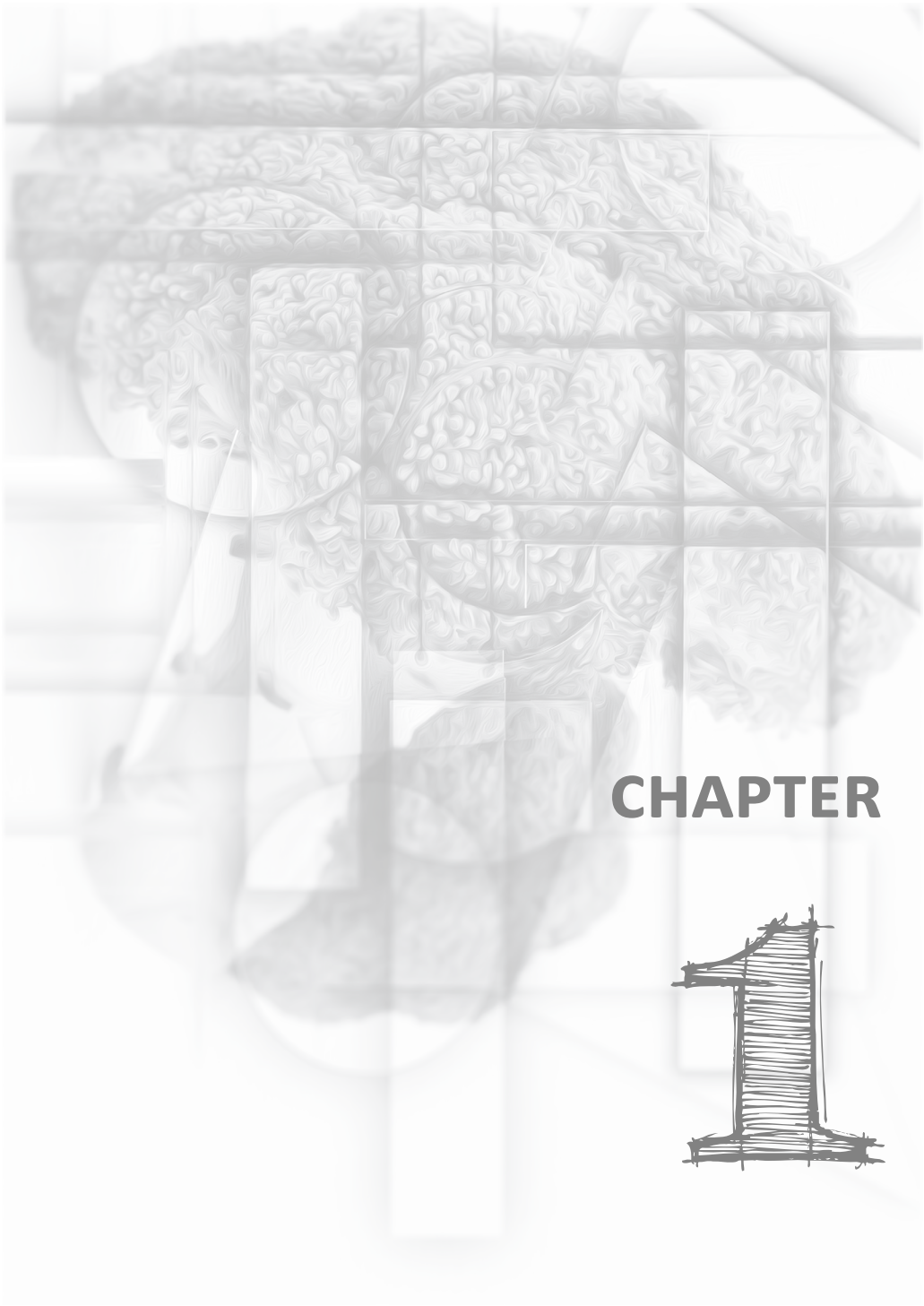
Food processing and health: a case on the glucosinolate - myrosinase system in dried broccoli,
162 pages.

PhD thesis, Wageningen University, Wageningen, NL (2013)
With references, with summaries in English and Dutch

ISBN 978-94-6173-800-4

Table of contents

General Introduction



CHAPTER

Food quality

Food quality refers to the combination of attributes that make food acceptable for consumers. The understanding of the quality concept is crucial to food technologists to control during the production and predict a certain level of quality in the final product. The quality concept is multidimensional, containing subjective and objective elements. While consumers do not consciously analyse all the elements of food quality separately but rather give an integrated judgement, food technologists have to disentangle the quality elements to control them during production. To simplify the quality concept to food technologists, food quality attributes can be divided in extrinsic and intrinsic factors. The extrinsic factors are related to religious and cultural aspects or the way food is produced, *i.e.* the use of pesticides, genetically modified ingredients, or organic production. The intrinsic factors are inherent to the product itself, *i.e*., sensory properties such as appearance, texture, flavour and factors related to food safety in order to prevent microbiological and toxicological contamination (Verkerk *et al.*, 2009). During the last decades health attributes, such as nutritional and health-promoting values, have become equally (if not more) important as sensorial attributes. Health attributes like bioactive compounds in horticultural crops (*e.g.* glucosinolates, polyphenols and carotenoids) have led to the development of a new image of horticultural product quality.

To satisfy the dynamic expectation of consumers a producer needs to design the production according to the needs and taste of the market. Therefore food quality is not a static property but it changes. Changes can be quantified via kinetic modelling. Kinetic modelling is a useful tool that can help producers to design food processes to obtain final products with certain quality attributes. Kinetic modelling can be used to control and predict quality attribute variation throughout the food chain.

The health quality attributes that will be considered in this thesis are the occurrence of a class of healthy compounds in *Brassica* vegetables called glucosinolates.

Health effect of vegetables and fruits

The protective role of vegetables and fruit consumption against various chronic diseases has been highlighted in several epidemiological studies and the consumption of fruit and vegetables is recommended by dietary guidelines (Steinmetz *et al.,* 1996; Liu, 2003; Martin, 2012).

The health effects of vegetables and fruits could be explained by the occurrence of a variety of vitamins, minerals, fibres and many classes of bioactive compounds from plants, called phytochemicals. Phytochemicals are often responsible for colour and other organoleptic properties of vegetables and fruits. The term is used to refer to compounds that may have biological effects. For instance carotenoids, present many fruits and vegetables, protect against cardiovascular disease and some types of cancers (Cooper *et al.*, 1999; Martin, 2012). Anthocyanins that occur in purple broccoli, egg plants, plums, cherries and many types of berry, have a protective effect against cardiovascular disease and age-related degenerative diseases associated with the metabolic syndrome and with inhibition of some stages of tumour development (Wang & Stone 2008; Martin, 2012). Isoflavones that occur in soy products may reduce the incidence of breast and prostate cancers, osteoporosis, cardiovascular diseases (Munro *et al.*, 2003; Martin, 2012).

Although there is a general consensus towards the protective role of vegetables and fruit for various chronic diseases, the outcomes of epidemiological studies sometimes conflict due to the multiplicity of confounding factors that interfere with interpretation of the outcomes (Steinmetz *et al.*, 1996). For instance, to study the association of cancer incidence and consumption of a vegetable that contains a compound that shows a certain bioactivity *in vitro*, an epidemiological study can only consider variables like consumption frequency, portion size, the way it is consumed (raw or cooked) and the consumer habits (like sporting, smoking, etc.). In such a study, the real intake of that compound is an unknown variable. It is demonstrated that many steps in the food production chain, like cultivation, storage, processing of vegetables can dramatically affect the levels and thus intake of phytochemicals (Dekker & Verkerk, 2003). To investigate the effect of consumption of vegetables that contain a certain compound, epidemiological studies should be integrated with *in vitro* and *in vivo* studies to test the bioactivity and bioavailability of that compound, to have a better picture of the health effect resulting from its consumption.

Food processing

The main purpose of industrial food processing is to produce safe and high quality food as demanded by consumers (Luning & Marcelis, 2009). Primary aims of food processing are the inactivation of food-borne pathogens, natural toxins and enzymes to prolong shelf-life. The beneficial aspects of food processing for consumers are various. Processing like drying, canning and freezing allow consumption of food independently from the seasonal availability. Besides, processing gives the opportunity to buy ready-to-eat and semi-prepared foods, *e.g.* microwavable frozen meals.

However, processing can also damage food quality. Often thermal treatment such as sterilization, leads to the loss of essential nutrient and phytochemicals mainly caused by thermal degradation or oxidation. Thermal treatments can also lead to the formation of undesired harmful products, *e.g.*, acrylamide in potatoes upon frying and heterocyclic amines in meat upon grilling. Also, sensorial quality attributes can be affected by processing such as sterilization and drying, *e.g.*, changes in colour, texture and flavour that have a negative effect on food organoleptic properties.

Types of processes

Food processing can be defined as the transformation of raw ingredients in food. Countless processes are implemented by the food industry to this end which include fermentation, fractionation, pasteurization, sterilisation, cooking, baking, frying, drying, packaging and the like. Processes that are based on heat treatments such as pasteurization and sterilization are of major importance in the food industry.

Alternative processes have been developed over the last decades to preserve the nutritional quality of food in the same time ensuring the adequate level of safety and organoleptic quality. One such example is pulsed electric field treatment that involves the application of pulses of high voltage to foods placed between 2 electrodes. This technology is considered superior to traditional thermal treatments because it avoids or greatly reduces the detrimental changes of the sensory properties of foods and the formation of undesired compounds. However, spores and enzymes are not inactivated (Raso & Heinz, 2007).

Another new technology makes use of high pressure to inactivate microorganisms, toxins and enzymes. By applying high pressure there is no formation of undesired compounds and the nutritional values are largely maintained, but also desirable flavour compounds generated by heat, are not formed.

Drying is another major process applied to food. In general, the main advantage of drying processes is the extension of shelf life with the possibility of storing dry food at room temperature, allowing consumption of vegetables out of season (Cohen & Yang, 1995). In most cases, drying is carried out by applying high temperatures to dehydrate food, *e.g.*, air drying. Air drying is a very common drying technology to dry foodstuffs. It is performed in ovens where hot air flow surrounds the product causing evaporation of water. The drying temperature ranges between 40 °C and 80 °C, and air flow velocity ranges between 0.5 and 5 m/s (Aversa *et al.*, 2006). The use of high temperatures combined with a high air flow velocity leads to structural, nutritional and biological change in the final product that is, by the way, negatively perceived by consumers. However, drying can also be carried out by applying low pressure, *i.e.* freeze drying. Among the drying methods, freeze drying can retain most of the nutritional properties of food, since it is performed at low temperatures, although it is not widely used in food industry due to its high operational costs.

Effect of processing on nutritional quality of vegetables

Food processing may either negatively or positively affect the nutritional quality of vegetables. In most cases, processing results in an adverse effect on the nutritional quality of vegetables. The most common effect is the loss of vitamins and phytochemicals upon heat treatment. The extent of the loss depends on the heat load applied, the kinetics of degradation of the target compounds and other process conditions, *e.g.* the oxygen level. Notable examples are the loss in vitamin C and glucosinolates in broccoli that will be discussed below. In other cases, nutrients can be lost by leaching during washing or blanching of the vegetables and exposure to water during cooking/processing. This also applies to domestic cooking incidentally. Another example will be specifically discussed in the present thesis, *i.e.* enzyme inactivation. Cutting is also known to impact nutritional quality of fruits and vegetables by exposing the labile healthy compounds to oxygen or hydrolytic enzymes. In some cases, however, processing can have a positive effect on the nutritional quality of vegetables (Van Boekel *et al.*, 2010). New compounds can be formed or the bioavailability of

healthy compounds upon processing can be increased. For instance, an increase of carotenoids concentration is reported when cooking tomatoes due to the thermal disruption of the non-covalent association between carotenoids and proteins present in the cell chloroplasts with the subsequent better extractability and digestibility of free carotenoids (Van het Hof *et al.*, 1998; Pellegrini *et al.*, 2010). Studies reported that the plasma folate response is higher when consuming minced spinach than intact leaves, suggesting that the food matrix in which the folate is entrapped plays a role in folate bioavailability (Castenmiller *et al.*, 2000).

Food process optimization

All in all, food processing can reduce nutritional values, especially thermal processing, therefore, it is preferable to minimally process fruits and vegetables and it is always mandatory to optimize the process conditions so to limit as much as possible the detrimental effect on nutritional quality. However, food processing can be optimized to achieve any desired quality attribute, the retention of health promoting compounds clearly being the most important.

The standard procedure for process design starts from the selection of raw materials and ends with the final product, passing through the sequence of unit operations. To optimize the process condition to achieve a final product with the desired quality attributes, static optimization is often applied, in which the optimal constant settings of the operation variables during a complete production run are selected (van Boxtel *et al.*, 1992).

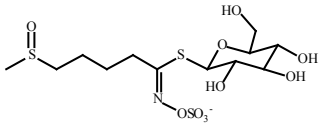
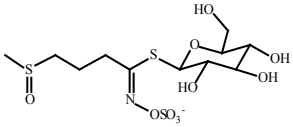
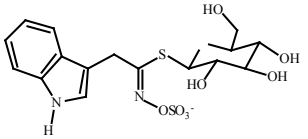
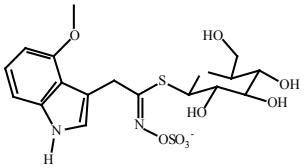
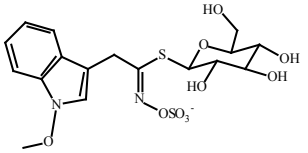
With the aid of the reverse engineering approach, the food process can be analysed starting from selecting required final product quality attributes, backward to raw material characteristics and process conditions that can influence the selected quality attribute in the final product (**Chapter 2**). To optimize the process condition to achieve a final product with the desired quality attributes, the application of dynamic process optimization is recommended. Food processing is a dynamic process that involves changes of the product characteristics as function of time. Dynamic optimization leads to optimal path of the control variables (trajectories) as a function of time.

In the present thesis an example of dynamic food process optimization based on the glucosinolate-myrosinase system in broccoli during drying will be described and discussed.

Glucosinolate-Myrosinase system

Vegetables like broccoli, cauliflower, cabbage, Brussels sprouts, belong to the *Brassica* genus and are widely consumed. A healthy diet should include *Brassica* vegetables because these vegetables are rich in health-promoting compounds like ascorbic acid, soluble fiber, selenium, glucosinolates, etc. Among these compounds, glucosinolates (GLs) have been extensively studied in the past decades. GLs are secondary metabolites (β-thioglycoside N-hydroxysulphates linked with a sulphur β-D-glucopyranose) that can be classified as aliphatic, aromatic or indolic depending on their side chain (Figure 1.1 and Table 1.1) (Fahey *et al.*, 2001). GLs are hydrolysed by a group of endogenous β-glucosidases termed myrosinase (thioglucoside glucohydrolase, EC 3.2.3.147). Myrosinase (MYR) is stored separately from GLs in the plants, but will mix with GLs upon tissue damage (Kissen *et al.*, 2009). Hydrolysis of the thioglucosidic bond by myrosinases releases an aglycone that can either rearrange into an isothiocyanate or be converted to other products such as nitriles, epithionitriles or organic thiocyanates (Figure 1.1) depending on the presence of specific proteins and certain structural prerequisites (Fahey *et al.*, 2001). Most breakdown products are volatile and lipophilic, which allows them to evaporate from damaged tissue and to enter living cells. Isothiocyanates (ITCs) have been demonstrated to be toxic to a wide range of organisms, including microorganisms, nematodes, and insects but they are potentially healthy when absorbed by human beings. ITCs are thought to be accountable for reducing the risk of several types of cancer (Verhoeven *et al.*, 1997). Broccoli is among the *Brassica* vegetables the richest in glucoraphanin (4-methylsulfinylbutyl glucosinolate) (Table 1.1), an aliphatic GL which corresponding ITCs have been identified as a particularly potent anticarcinogen in humans and animals (Juge *et al.*, 2007). Besides glucoraphanin, many other GLs have been identified in broccoli: glucoiberin (3-methylsulfinylpropyl glucosinolate), glucobrassicin (indol-3-ylmethyl glucosinolate), 4-methoxyglucobrassicin (4-methoxy-indol-3-ylmethyl glucosinolate) and neoglucobrassicin (N-methoxyindol-3-ylmethyl glucosinolate).

Table 1.1: Trivial names, chemical names and structures of the main glucosinolates present in broccoli analysed in research presented in the thesis.

Trivial name	Chemical name	Structure
Glucoraphanin	4-methylsulfinylbutyl-glucosinolate	
Glucoiberin	3-methylsulfinylpropyl-glucosinolate	
Glucobrassicin	indol-3-ylmethylglucosinolate	
4-methoxy-glucobrassicin	4-methoxyindol-3-ylmethyl-glucosinolate	
Neo-glucobrassicin	N-methoxyindol-3-ylmethyl-glucosinolate	

Effect of drying processes on the Glucosinolate-Myrosinase system

Food processing often leads to reduction of the GLs content, MYR activity, hence to the ITCs formation and intake. Processes that make use of high temperature as cooking can:

a. Partially or fully inactivate MYR;

b. Damage the vegetable matrix and cause leaching in cooking water of GLs and ITCs (when formed);

c. Thermally degrade GLs.

For instance, domestic cooking such as boiling leads to substantial GLs leaching and degradation, and MYR inactivation depending on the sort of vegetable, temperature, cooking time and ratio vegetable/water.

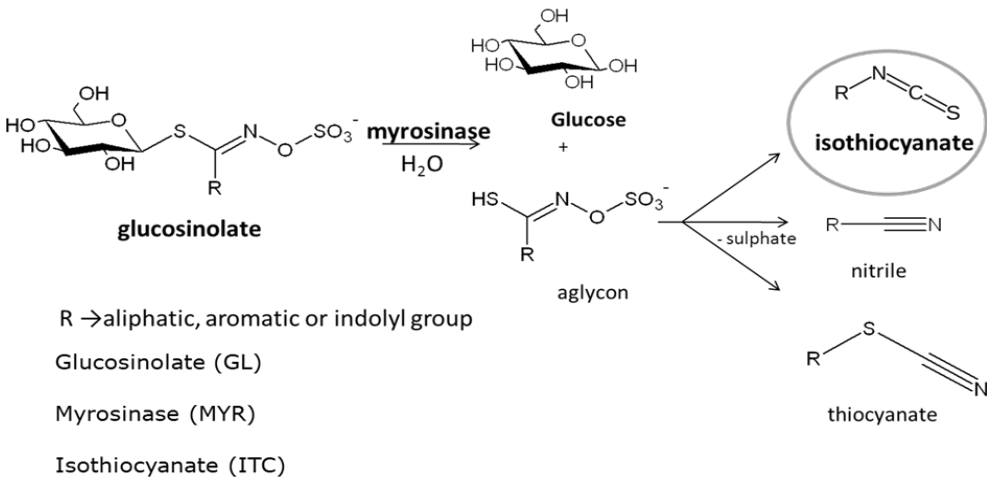


Figure 1.1: General glucosinolates structure, hydrolysis reaction and breakdown products.

Air drying can affect the health- promoting effect by reducing GLs content, MYR activity and ITCs formation due to the use of high temperatures combined with a high air flow velocity. Mild drying conditions are preferred to retain the health properties of broccoli. Nevertheless, mild conditions conflict with the drying efficiency. Jin *et al.* (2012) reported that by following optimal temperature drying trajectories, to air dry broccoli, glucoraphanin was retained in combination with an increased energy efficiency. The drying process was optimized by using mathematical models. Mathematical modelling is a very useful tool for process design and it was used in this thesis to optimize the air drying.

Isothiocyanates absorption and excretion in human beings

In the human body, after ingestion and absorption, ITCs are conjugated to glutathione, further metabolized to ITC conjugates, and subsequently excreted in urine (Shapiro *et al.*, 1998, Mennicke *et al.*, 1988, Vermeulen *et al.*, 2008). ITCs absorption can be assessed measuring the ITC conjugates excreted in urine (Shapiro et al, 1998; Vermeulen *et al.*, 2008). The opportunity to monitor the ITCs conjugate excretion has triggered many *in vivo* studies in which the effect of consuming raw (with active MYR) and cooked (with inactive MYR) *Brassica* vegetables on ITCs absorption was studied. One of the most interesting finding of these studies is the evidence of the MYR-like activity of the enteric microflora of human beings. GLs that have not been hydrolysed by the plant MYR, are hydrolysed upon digestion, by the MYR-like activity of the enteric microflora, even though to a lesser extent (Shapiro *et al.*, 1998) (Figure 1.2).

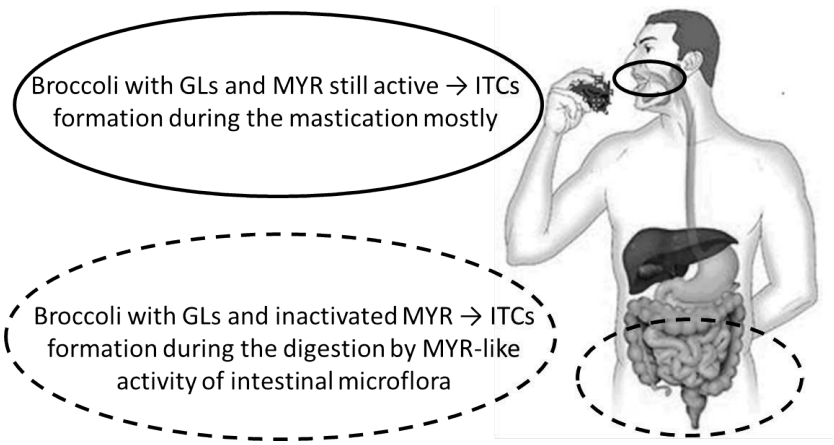


Figure 1.2: Two glucosinolates (GLs) hydrolysis path. Myrosinase (MYR), isothiocyanates (ITCs).

The factors that mostly affect the ITC intake are (1) genetic factors like initial GLs concentration and MYR activity and occurrence of MYR cofactors, like vitamin C and magnesium chloride; (2) environmental factors like cultivation practices; (3) processing conditions like industrial and domestic cooking; (4) inter-individual variation of consumers like mastication extent, meal composition, intestinal microflora type (Verkerk *et al.*, 2009).

Objective and outline of the thesis

This research is part of a broader project called "Energy-efficient Drying of Healthy Food Products", aimed to improve the quality of dry foodstuffs focusing on retention of healthy compounds while increasing sustainability of the drying process by reducing the energy consumption. Along with the present PhD project, other two PhD projects have run on complementary topics. One project investigated the energy-efficient low-temperature drying using adsorbents, and another project worked on the optimization of drying conditions to retain healthy compound by using simulation models. The GLs-MYR system in broccoli (*Brassica oleracea* var. *italica* was selected as case study. The research described in this thesis, which focused on the effect of water content on thermal kinetic degradation of GLs and MYR, can be regarded as the connection between these two PhD projects. GLs concentration and MYR activity can be drastically reduced by food processing like air drying. Nevertheless, air drying is a widely used and useful process. Hence, there is a need to design air drying processes aimed to retain GLs and MYR activity. The aims of this study were (1) to kinetically model the effect of temperature and water on GLs degradation and MYR inactivation. These models were used to optimize air drying conditions to obtain a broccoli product with certain GLs levels and MYR activity. (2) to investigate *in vivo* the absorption of ITCs after consuming dried broccoli products with different GLs, MYR and ITCs profiles, as measure for the health related quality attributes.

Mathematical kinetic modelling has been used to simplify and predict complex reactions that occurs during food processing (Van Boekel, 2009). With the aid of kinetic modelling, thermal degradation of GLs and MYR inactivation during air drying, was estimated. Then, according to these kinetic models, air drying conditions that totally or partially prevent the loss of GLs and MYR were estimated and validated. A human intervention study was performed to study the actual absorption of ITC after consuming dehydrated dried broccoli products with different GLs, MYR and ITC profiles.

In **Chapter 2** an approach for process design to control the degradation of GLs, MYR and formation of ITCs in broccoli during air drying and air drying pre-treatment is presented. Dry broccoli products with different GLs, MYR and ITCs profiles and in turn with different potential health benefits are described. Depending on the

characteristic of the final product, different drying scenarios, with different drying and pre-drying conditions, are proposed.
Then, to optimize drying process conditions to produce dried broccoli with specific GLs and MYR profiles, thermal degradation and inactivation kinetics of GLs and MYR were studied. During drying broccoli is subjected to changes in water content and temperature which affect the occurrence of GLs and MYR. Hence, the influence of water content and temperature on GLs degradation and MYR inactivation kinetics was studied in **Chapter 3** and **Chapter 4**, respectively.

Based on the kinetics of GLs degradation and MYR inactivation, optimized temperature drying trajectories were obtained that totally or partially prevent GLs and MYR loss. **Chapter 5** describes the application of the optimized air drying trajectories by comparison with air drying at constant conditions and their impact on GLs and MYR.

To investigate *in vivo* the effect of broccoli products with different GLs, MYR and ITC profiles, in **Chapter 6** the actual ITC absorption (excretion kinetics and bioavailability), resulting from the consumption of these broccoli products, was systematically and quantitatively studied.
In the final chapter (**Chapter 7**), the main findings and their implications are summarized and discussed. Moreover, suggestions for future research are presented.

A research approach for quality based design of healthy foods: dried broccoli as a case study

Oliviero T.
Verkerk R.
Dekker M.

Trends In Food Science & Technology
(2013) Volume 30, Issue 2, Pages 178–184

CHAPTER

Abstract

An approach for process design based on optimization of product quality attributes is presented. Adsorption drying of broccoli with retention of its health benefits is taken as an example to illustrate the approach. Related to its content of glucosinolates, broccoli has a high potential to reduce the risk of some types of cancer. Three products are described with different glucosinolates, myrosinase and isothiocyanates related health profiles. These products require different drying scenarios with specific optimization targets. This viewpoint paper presents a case on quality based process design as an example for broader application onto food products.

Introduction

Conventional approaches for food process optimization are based on maximization of process efficiency by increasing the yield and minimizing the overall costs. The quality of a product is a necessary requirement to make consumers buying the product, but it is not the main aim of the conventional food process optimization. Nowadays consumers perception of product quality is increasingly important for the market success of products. Health attributes of products are becoming more and more important since there is a growing awareness of consumers that a healthy diet positively affects their well-being and can reduce their risk for certain diseases (International Food Information Council Foundation, 2011; Ragaert *et al.*, 2004; Wandel, 2007). Moreover there is a growing interest in sustainable technologies that can reduce the extent of environmental pollution. Therefore new food process optimization approaches where the main goal is the quality of a final product and where limitation of energy consumption is also considered, are necessary. With the aid of reverse engineering, the whole food process can be examined starting from final product properties backward to raw material selection and process conditions that can influence the quality of the final product (Hadiyanto *et al.*, 2008). The first step is to determine which properties in a final product are required and then investigate the process conditions that enable to reach those quality criteria.

In this article a low energy air drying process of broccoli is discussed with retention of the health benefits of broccoli as a quality requirement in the production of dried broccoli. Broccoli is rich in flavonoids, vitamins, mineral nutrients and glucosinolates (GLs) (Moreno *et al.*, 2006). In particular, the occurrence of GLs have been associated with the prevention of some types of cancer (Verhoeven *et al.*, 1997) and hence the need to control for their retention. The aim of this optimization approach is to control the degradation of GLs, myrosinase (MYR) and formation of biological active isothiocyanates (ITCs) during pre-treatments and adsorption drying process of broccoli. For comparison the effect of hot air drying and conventional water blanching pre-treatment, widely used in the food industry, are discussed. Three dry broccoli products with different GLs, MYR and ITCs profiles and in turn with different potential health benefits are described. A research approach to optimize three drying scenarios is presented in order to obtain these three products with different potential health benefits.

Hot air drying

Drying is a common process to remove moisture from foods, in order to prevent microbiological growth and enzyme activity, thereby enhancing the storage stability. By extending the shelf life without the need for refrigeration or freezing, drying allows the consumption of vegetables out of season (Cohen & Yang, 1995).

Various drying technologies are used worldwide, the most applied industrial technique is forced convection by hot and dry air (Aversa *et al.*, 2006). Examples of commercially important dried foods are coffee, milk powder, sultanas and other fruits, pasta, flours (including bakery mixes), beans, pulses, nuts, breakfast cereals, tea and spices. Dried vegetables can be consumed directly as chips, or be reconstituted for a cooked side dish, or used in soups, sauces, stews, casseroles and stuffings.

Hot air drying is one of the common technology performed in ovens where hot air flow surrounds the product causing evaporation of moisture. The drying temperature ranges between 40°C and 80°C, and air flow velocity ranges between 0.5 and 5 m/s (Aversa *et al.*, 2006). The use of high temperatures combined with a high air flow velocity leads to physical, chemical, biological and nutritional change, which negatively affect the quality of the final product (Perera, 2005; Sablani, 2006). Specifically, the sensory, nutritional and health-promoting value of dried vegetables have to be considered. Several effects of drying conditions on vitamins, amino acids and minerals have been described (Benali, 2004; Kunzek & Vetter, 1999). Moreover drying is always combined with other treatments such as washing, chopping, water blanching and grinding that may affect the quality of the final product. When optimizing the product quality all processes should be considered for their effect on the quality attribute. Adsorption drying might be a suitable option for preserving the health effect of broccoli with a limited ecological impact deriving from low energy consumption.

Adsorption drying

Adsorption drying is a new technology that can be classified as air drying. The advantage is that it can dry at lower temperature by using an adsorbing material. This material adsorbs moisture from the air flow regenerating it during drying. For this reason it allows lower drying temperatures and lower energy consumption, while maintaining a high driving force for drying due to the low water activity of the regenerated air (Atuonwu *et al.*, 2011).

The adsorption drying process consists of a dryer, a heat source and a zeolite adsorption/regeneration system. Ambient air passes through a heater, then through a zeolite adsorber bed, where it is dehumidified and ready to be used for drying the product. Both humidity reduction and temperature rise (40/50°C) increase the capacity of the air to evaporate moisture from the product. Finally the spent zeolite is regenerated using hot air obtained by passing ambient air through a heater (Atuonwu *et al.*, 2011).

Product properties related to health benefits of broccoli

GLs are a group of secondary plant metabolites (β-thioglycoside N-hydroxysulphates linked with a sulphur β-D-glucopyranose) that occur in *Brassica* vegetables. Depending on the side chain GL can be classified as aliphatic, aromatic or indolic (Fahey *et al.*, 2001). The MYR enzyme (thioglucoside glucohydrolase, EC 3.2.3.147) occurs in *Brassica* vegetables, but is physically separated by GLs in intact tissue (Kissen *et al.*, 2009). When the plant tissue is damaged, cells are broken down releasing MYR and GLs. The consequence is that MYR catalyses the hydrolysis reaction of GLs forming unstable compounds that spontaneously break down into a range of reactive chemicals including ITCs (Fahey *et al.*, 2001). ITCs are thought to be accountable for decreasing the risk of several types of cancer (Verhoeven *et al.*, 1997). Another characteristic of ITCs is their volatility that makes ITCs prone to escape from the plant matrix during the processing (Fenwick *et al.,* 1983b).

Many postharvest treatments may cause the decrease of GLs, MYR and ITCs including domestic cooking (Dekker *et al.*, 2000). During cooking several processes take place. High temperatures cause GLs degradation, MYR inactivation and cell lysis. When GLs

and MYR are not totally depleted, the cell lysis allows the release of these compounds and the formation of breakdown products such as ITCs. At high temperatures ITCs can degrade or evaporate depending on their volatility. Moreover boiling of vegetables causes the leaching of GLs into the cooking water, thus impacting the total intake of these healthy compounds (Dekker *et al.*, 2000).

It is thus evident that the health effect derived by the consumption of *Brassica* vegetables strongly depends on the processing conditions. In a product where both MYR and GLs are retained, ITCs formation occurs during mastication, which makes ITCs readily available in the gastrointestinal (GI) tract for exerting its beneficial effects on health. In a product where MYR is inactivated, which is the case for most *Brassica* vegetables in our diet, ITCs formation occurs in the gut by means of MYR-like activity of gut microflora (Shapiro *et al.*, 1998). Although the conversion occurs to a lower extent, it is apparently still enough for observing health benefits, as several epidemiological studies have shown (Traka & Mithen, 2009). In a product where ITCs have been formed during processing, ITCs could be absorbed from our body promptly (Shapiro *et al.*, 1998). But in this case, the actual intake depends on the extent of ITCs formation and loss during the preparation.

Effect of conventional drying on glucosinolates and myrosinase

During hot air drying of broccoli several mechanisms could be responsible of changing the health promoting effect of the product. GLs could be broken down enzymatically by MYR and chemically by elevated temperatures and MYR can be inactivated. Moreover, cellular structures can be damaged and ITCs formed, but then they can be thermally degraded (Van Eylen *et al.*, 2007) or evaporated from the product (Jang *et al.*, 2010; Mari *et al.*, 2008). However few data are available so far about the effect of conventional hot air drying on GLs MYR and ITCs.

Bailey *et al.* (1961) investigated the formation of volatile sulphur components of minced fresh cabbage and reconstituted dried cabbage either with water or with water plus standard MYR. In the minced fresh product, five ITC compounds were detected. In rehydrated product (with water only) no volatile ITCs were detected, whereas rehydrated product with water and added myrosinase yielded ITCs, indicating the retaining of parent GLs and destruction of MYR in the drying process. A drastic thermal treatment

might explain MYR inactivation, but the drying temperature was not reported by the authors. According to Daxenbichler *et al.* (1977) the MYR activity was not lost when cabbage or Brussels sprouts were dried overnight at 50°C in a forced-draft oven. Indeed, after rehydrating the cabbage the enzymatic hydrolysis of the GLs occurred.

Mrkic *et al.* (2010) investigated the combined effect of water blanching and subsequent hot air drying (50-100°C range) on indolic GLs in broccoli. During the drying step, major losses of GLs occurred at 100 °C, which was the highest temperature used. A clear relation between the change of total and individual GLs and drying temperature and air velocity was not found. However the best drying conditions to retain the total GLs content were at 50°C with an air velocity of 1.2 m s^{-1} and at 60°C with 2.25 m s^{-1}.But the water blanching treatment, applied before drying, caused already around 64% decrease in GLs content, likely due to the leaching of GLs in blanching water (Mrkic *et al.*, 2010).

Therefore the treatment that potentially affects the GLs and MYR content most is water blanching, causing a higher loss of healthy compounds than the drying process itself. However, this treatment is important to produce good quality dried vegetable in a relatively short time. Basically two main processes take place during water blanching: (1) partly destruction of vegetable tissue, because of cells damage, favoring a much faster water loss, (2) reduction of the microorganism content and inactivation of enzymes such as polyphenoloxidase and peroxidase (Sunjka *et al.*, 2008).

The same purposes could be achieved by replacing water blanching with steam blanching treatment (Ndiaye *et al.*, 2009; Rossi *et al.*, 2003). Advantages of using such treatment are: prevention of GLs leaching into cooking water and retaining of part of MYR activity by steam blanching for short time. Indeed, steam blanching can be the suitable option as drying pre-treatment. A complete overview on GLs content, MYR activity and cell lysis according to temperature changes inside broccoli during steam blanching, was showed by Verkerk *et al.* (2010).

By optimizing the pre-treatment and drying conditions one can convert dried vegetables into a more healthy final product.

In this article an optimization approach for drying of broccoli is discussed, considering the changes in the composition of the vegetable tissue. Three potential broccoli products which contain different GLs, MYR and ITCs profiles are described. These products would exert varied health benefits depending on whether GLs are hydrolysed during processing or in the GI tract after consumption.

Desired product properties for health benefit of broccoli

Different product concepts can be envisaged based on the knowledge of the mechanism of action of GLs on human health. Three dry broccoli products produced by combining steam blanching and air drying have been designed. These three broccoli products will differ in GLs, MYR and ITCs profiles and in turn with different potential health benefits. According to the different profiles, GLs and ITCs will be metabolized either in lower GI tract (colon), in the case the product contains high GLs and no MYR (Figure 2.1), or the upper gastrointestinal tract, in the case of broccoli containing high GLs and high MYR (Figure 2.3), or readily available ITCs (Figure 2.2).

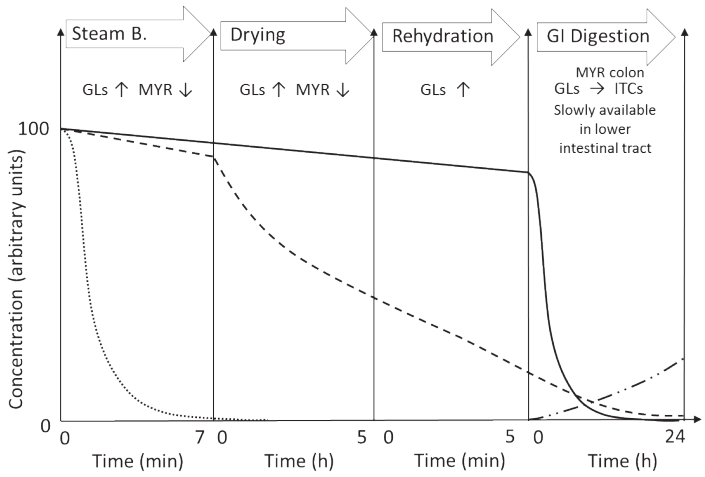


Figure 2.1: Schematic illustration of the first broccoli product concept. Intact cells (▬ ▬ ▬); isothiocyanates (▬ . . .); total glucosinolates (▬▬); myrosinase (.....). High (↑) and low (↓) concentration of glucosinolate (GLs), myrosinase (MYR) and isothiocyanates (ITCs) during the adsorption drying process (steam blanching (Steam B.) and drying), rehydration and gastrointestinal (GI) digestion of the first broccoli product.

First product concept

The first broccoli product concept is aimed at containing high GLs levels, but no MYR (Figure 2.1). In this case ITCs formation would take place during GI digestion of broccoli due to the MYR-like activity of microflora of intestinal tract (Shapiro *et al.*, 1998). To produce such a product, steam blanching should inactivate MYR, while retaining GLs. Drying should not lead to degradation of GLs.

Second product concept

The second broccoli product concept is aimed at containing readily available high ITCs level for direct absorption into the gut (Figure 2.2). For this purpose GLs conversion by MYR should occur prior or during drying. GLs and MYR should not be lost before this conversion and ITCs should not be lost during drying.

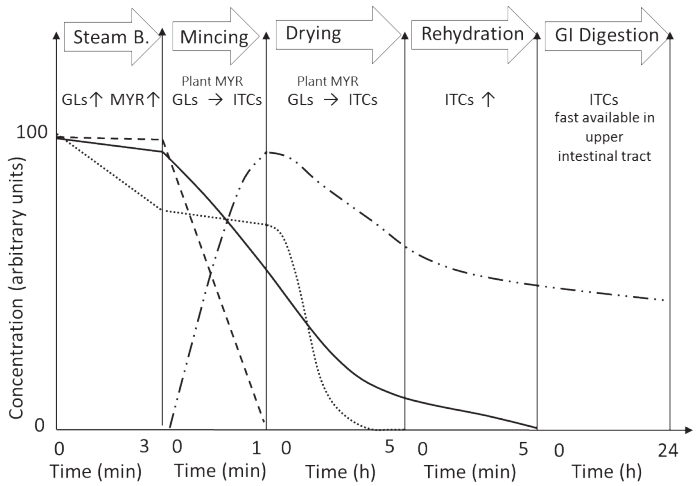


Figure 2.2: Schematic illustration of production of the second broccoli product. Intact cells (▬ ▬ ▬); isothiocyanates (▬ . . .); total glucosinolates (▬▬); myrosinase (.....). High (↑) and low (↓) concentration of glucosinolate (GLs), myrosinase (MYR) and isothiocyanates (ITCs) during the adsorption drying process (steam blanching (Steam B.), mincing and drying), rehydration and gastrointestinal (GI) digestion of the second broccoli product.

Third product concept

The third broccoli product is designed to have high GLs content and MYR activity (Figure 2.3). GLs and at least part of the MYR activity should be retained during the pre-treatment and drying process. ITCs formation should occur during domestic rehydration and mastication of final product, thus ITCs would be ready to be absorbed into the GI tract. Therefore ITC formation must be prevented during pre-treatment and drying and minimized during rehydration for maximizing its release during GI digestion.

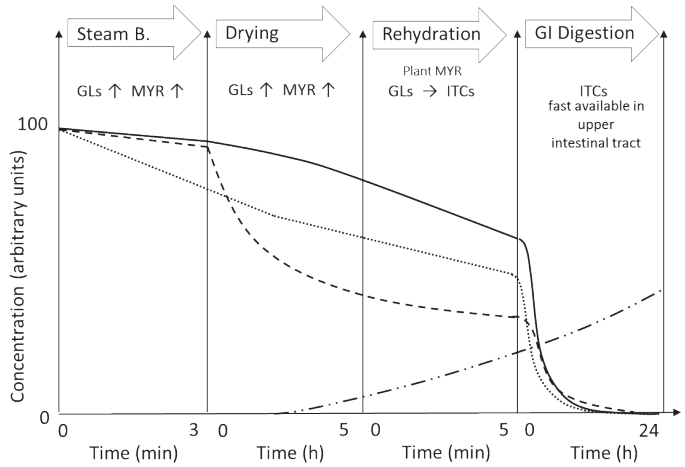


Figure 2.3: Schematic illustration of production of the third broccoli product. Intact cells (– – –); isothiocyanates (— . .); total glucosinolates (———); myrosinase (.....). High (↑) and low (↓) concentration of glucosinolate (GLs), myrosinase (MYR) and isothiocyanates (ITCs) during the adsorption drying process (steam blanching (Steam B.) and drying), rehydration and gastrointestinal (GI) digestion of third broccoli product.

How to optimize process conditions?

In the first broccoli production scenario (final product with high in GLs and no MYR), a steam blanching pre-treatment would be preferred to water blanching to reduce the leaching of GLs into blanching water and it should last long enough to ensure the complete inactivation of MYR. According to literature data no residual MYR activity is detected in cabbage and broccoli after approximately 7 minutes of steam blanching (Rungapamestry *et al.*, 2006; Verkerk *et al.*, 2010). In the same time GLs content does not change significantly in cabbage (Rungapamestry *et al.*, 2006) and even increased in broccoli (Verkerk *et al.*, 2010), due to an increase in GLs extractability. Therefore a steam blanching pre-treatment for approximately 7 minutes may be suitable in this broccoli production scenario.

After steam blanching broccoli should be dried at mild temperature to retain GLs. As showed by Mrkic *et al.* (2010), by air drying blanched broccoli at 50 °C around 86% of GLs were retained.

In the second broccoli production scenario (final product with high ITCs content), broccoli should undergo a mild steam blanching in order to keep high GLs levels as well as to prevent the total MYR inactivation.

Verkerk *et al.* (2010) detected approximately 60% of myrosinase activity and 120% of extractable GLs content in broccoli after 3 minutes of steam blanching. Thus, after steam blanching at these conditions, a part of MYR could be still active and GLs concentration would be still high. Then, to allow MYR to catalyze ITCs formation, a mincing step to destroy cell walls should be applied. Depending on ITCs volatility and thermostability, these compounds may be lost to different extents during drying, due to temperature and air flow velocity of the drying. It is known that different ITCs would show a different volatility (Vermeulen *et al.*, 2009). Temperature was also found to have a great effect on ITCs degradation. For instance higher sulforaphane (the corresponding ITC of glucoraphanin, an aliphatic GLs) degradation products were detected in sulforaphane water solutions (heated at 100°C for 1 h compared to sulforaphane water solutions heated at 50°C for the same time (Wang *et al.*, 1999). In consequence the drying should be performed at mild temperature and mild flow velocity (40-50 °C and 0.5-1.5 m/s). Domestic rehydration of this product should be performed by using mild conditions as well to maximise the retention of ITCs for direct absorption into the upper GI tract.

In the third broccoli production scenario (final product with high in GLs and MYR), a mild steam blanching and mild drying process as described for the second scenario should be used to preserve MYR activity and GLs. Unlike the second scenario, the cell damage should be avoided to prevent ITCs formation during processing and during domestic rehydration. Actually, during domestic rehydration, ITC formation would occur to a certain extent but mainly it would occur during digestion in the upper GI tract.

In order to optimize processing conditions of the absorption drying technology to achieve the desired health beneficial characteristics of dried broccoli, research in the kinetics of several reactions is required.

During drying broccoli is subjected to changes in water content and temperature which may affect the physical structure and the content of MYR, GLs and ITCs. To design suitable drying conditions, investigations of the influence of water content and drying temperature on GLs stability, MYR activity and stability, ITC formation and stability, cells lysis and drying rate are necessary.

For this purpose broccoli samples with different water content have to be thermally treated at different times and temperatures. The kinetics of the effects on GLs, MYR, ITCs and cell lysis can be mathematically modelled to estimate the parameters of these reactions. The effect of water content on GLs thermal degradation has been already investigate by our research group (Oliviero *et al.*, 2012-**Chapter 3**). Broccoli batches with water content between 13% and 82%, in which MYR was inactivated, were heated at different temperature (from 60 to 120 ˚C) and GLs concentrations were monitored. The GLs thermal degradation was described by the first order kinetic. The sample with 13% showed the lowest degradation rate at heating temperature range 60–100 ˚C. On the contrary at 120 ˚C the GLs degradation rate of broccoli with 13% of water content, was the highest (Figure 2.4). The effect of water content on MYR thermal inactivation has been also investigated by our research group (Oliviero *et al.*, 2013- **Chapter 4**). Broccoli batches with water content ranged between 10% and 90% were heated at different temperature (from 40 to 70 ˚C) and MYR activity was monitored. The results showed that MYR inactivation rate increased with the increase of broccoli water content (Figure 2.5). The findings of these researches will be used as guideline to promote or to prevent the GLs and MYR degradation according to the water content and temperature changing that characterize the air drying process.

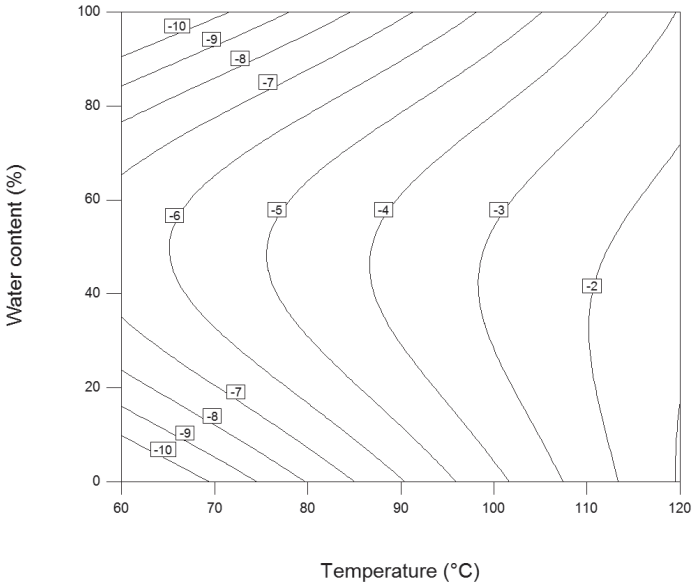


Figure 2.4: Contour plot of k values for glucoraphanin as function of temperature and water content of broccoli. Contour values represent ln(k) values (k is first order degradation rate in min^{-1}). Plot is fitted using data from Oliviero, Verkerk and Dekker (2012).

This integrated mathematical model will be the basis for optimizing the drying parameters: temperature, time and air velocity. Moreover the effect of water blanching and steam blanching conditions as a pre-treatment to drying on GLs, MYR, ITCs, cell lysis as well as duration of the following drying process has to be studied. Finally, by combining the information from those experiments and the integrated model, the whole drying process will be fine-tuned in order to achieve the desired characteristics in the final product.

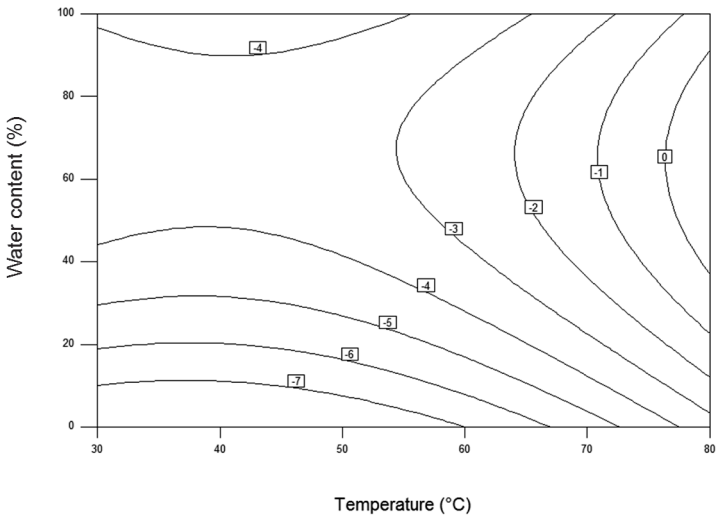


Figure 2.5: Contour plot of k values for myrosinase as function of temperature and water content of broccoli. Contour values represent ln(k) values (k is first order degradation rate in min-1). Plot is fitted using data from Oliviero, Verkerk, Van Boekel and Dekker (submitted).

Conclusions

There is a growing attention of the consumers towards the health-promoting properties of their diet, while there is a general concern on the energy use of food production systems. For these reasons to replace conventional hot air drying with adsorption drying, a low energy consumption drying that can retain the healthy compounds present in vegetables, is a sensible option. In this article a modern process optimization based on inverse engineering was proposed. The reverse engineering approach is a suitable tool to optimize the drying process, by first defining quality criteria of a desired product and then defining process conditions that allow to reach those criteria.

Drying of broccoli by using the adsorption air drying process was selected as case study in order to show how to apply this new optimization approach. As quality

criteria GLs, MYR and biologically active ITCs profiles were selected. Three dried broccoli concepts, each with different GLs, MYR and ITCs profiles and thus each with different health benefits were described. Following the reverse engineering approach three drying processes were proposed to develop these dried broccoli products.

Table 2.1: Potential applications of the reverse engineering approach to foodstuffs.

Food products	Key quality attributes	Process variables to monitor
Bread	✓ Volume ✓ Softness ✓ Colour ✓ Flavour	✓ Leavening conditions (time and temperature) ✓ Baking conditions (time and temperature)
Cookie	✓ Crispiness ✓ Colour ✓ Flavour	✓ Baking conditions (time and temperature)
Dried pasta	✓ Structure ✓ Colour ✓ "Al dente" characteristics during domestic cooking	✓ Drying conditions (time, temperature)
Fruit juices	✓ Colour ✓ Viscosity ✓ Enzymes inactivation ✓ Healthy compounds profile	✓ Pasteurization conditions (time, temperature, pressure)
Frozen vegetables	✓ Colour ✓ Enzymes inactivation ✓ Structure ✓ Healthy compounds profile	✓ Blanching conditions (time, temperature, pressure) ✓ Frozen conditions (time, temperature)

Moving from a conventional food process optimization approach, based on yield increase and minimization of the overall costs, to a new one which main goal is the quality of the final product should be a target for the food industries. A quality

driven design based on the reverse engineering approach is very useful tool for process optimization, which use can be extended to a very wide range of food processes. Table 2.1 shows some examples of how this approach may be applied in other food processing. For instance, regarding the production of cookies, the first step would be to select the key quality attributes, *e.g.* crispness, colour and flavour and then investigate the process variables, in this case the baking conditions, that affect these attributes. The same quality based design approach can be applied to the production of fruits juices by selecting the key quality attributes, *e.g.* the colour, the viscosity, the enzymatic activity and the occurrence of vitamins and antioxidants and then by identifying processing conditions that affect these parameters.

Acknowledgements

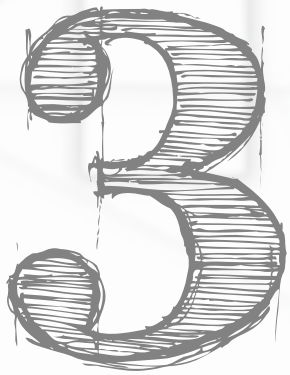
This work is supported by the Energy Research Program EOS of the Dutch ministry of Economics under project EOSLTe7043.

Effect of water content and temperature on glucosinolate degradation kinetics in broccoli (*Brassica oleracea* var. *italica*)

Oliviero T.
Verkerk R.
Dekker M.

Food Chemistry
(2013) Volume 132, Issue 4, 15, Pages 2037-2045

CHAPTER



Abstract

The effect of water content and temperature on glucosinolate thermal degradation in broccoli (*Brassica oleracea* var. *italica*) was investigated. Broccoli was freeze dried obtaining batches with water content between 13% and 82% (a_w from 0.32 to 0.99). These samples were heated at different temperatures (from 60 to 120°C) and glucosinolate levels were monitored. To rule out enzymatic breakdown, myrosinase was inactivated prior to heating. Degradation could be described by first-order kinetics for all glucosinolates and all water contents. In the temperature range 60-100°C the sample with 13% showed the lowest degradation rate, whereas at 120°C the degradation rate increased with the water content. This particular behaviour was reflected by the high activation energy value of the driest sample. Several hypotheses to explain the observed behaviour are discussed.

Introduction

Epidemiological studies have identified an inverse association between consumption of *Brassica* vegetables and the risk of some types of cancer (Hansson *et al.*, 1993; Zhang & Talalay, 1994; Bones & Rossiter, 1996; Lin *et al.*, 1998;). *Brassica* vegetables are rich in glucosinolates (GLs), a group of secondary plant metabolites. GLs are β-thioglycoside N-hydroxysulphates with a variable side chain which determines whether a glucosinolate is classified as aliphatic, aromatic or indolic (Fahey *et al.*, 2001). They can be hydrolyzed by myrosinase, an endogenous plant enzyme (thioglucoside glucohydrolase, EC 3.2.3.147), to form unstable compounds that spontaneously break down into a range of reactive chemicals including isothiocyanates. Isothiocyanates are thought to be accountable for decreasing the risk of several types of cancer (Verhoeven *et al.*, 1997).

GLs and myrosinase are physically separated from each other in intact vegetable tissue. The disruption of vegetal tissues caused by food preparation as well as by chewing of the vegetables releases the myrosinase that catalyses the hydrolysis reaction of GLs which in turn starts the cascade of reactions yielding isothiocyanates (Kissen *et al.*, 2009). A characteristic of isothiocyanates is their volatility, accounting for the typical flavour and odour of these vegetables (Fenwick & Heaney, 1983a; Fenwick *et al.*, 1983b).

Postharvest treatments such as storage and industrial/domestic processing are responsible for changes in GLs concentrations and in turn, for the isothiocyanates formation and their intake (Dekker *et al.*, 2000; Mithen, *et al.*, 2000; Verkerk *et al.*, 2009).

Domestic and industrial processing usually involve thermal treatment which could affect the health promoting compounds of *Brassica* vegetables. During heating many mechanisms take place: thermal degradation of GLs and breakdown products, enzymatic breakdown of GLs, myrosinase inactivation and leaching of GLs and breakdown product into cooking water (Dekker *et al.*, 2000). In most studies on the effect of thermal processing, the total loss of GLs is the result of many mechanisms occurring simultaneously. Leaching of GLs in the cooking water has been predicted by simulations to be the major factor which is responsible for GLs loss during boiling of vegetables (Verkerk, 2002). The effect of thermal processing on GLs content has

been investigated in many *Brassica* vegetables. The effect of boiling on GLs content in broccoli (Song & Thornalley, 2007), Brussels sprouts and cauliflower (Cieślik *et al.*, 2007) has been investigated as well as the effect of blanching and freezing on thermal degradation after boiling of broccoli (Pellegrini *et al.*, 2010; Rungapamestry *et al.*, 2008).

Data reported in literature, however, showed that the thermal degradation rate of GLs varies with the type of vegetable (Dekker *et al.*, 2009). These result suggest that the plant matrix plays an important role in determining the thermal degradation rate of GLs.

Oerlemans *et al.* (2006), focused the attention on thermal degradation of GLs in red cabbage. The authors blanched the samples by microwaving (without adding water) in order to inactivate the myrosinase and subsequently heated the samples at different temperature/time combinations in the absence of additional water to exclude leaching losses. The chemical degradation was described by first order kinetics and Arrhenius like temperature dependency. The parameter estimation showed that at temperatures below 110°C indole glucosinolates have a significantly higher degradation rate constant compared to aliphatic glucosinolates.

The studies mentioned above focused exclusively on the effect of processing conditions on the fate of GLs in fresh, frozen and blanched vegetables. No data are available on the effect of water content and/or water activity on the thermal degradation rate of GLs in vegetables. This is a major point since drying technologies give a great opportunity to extend the shelf life of the vegetable products by reducing a_w. Most of those technologies make use of hot air for the removal of water, This can have a significant impact on the GLs content of the final product (Mrkic *et al.*, 2010). Drying options that make use of low temperature and low pressure (freeze-drying) often retain more of the original flavour, texture and health promoting compounds. Those techniques, however, are much more expensive, time and energy consuming than those using hot air. Insight on the effect of water content/a_w on the degradation rates of GLs is thus mandatory for the optimization of the air drying technology conditions that allow to obtain the desired products retaining high GLs content of the fresh vegetables and in the same time limiting the energy consumption. Water content and water activity can have multiple effects on the rate of reactions in foods (Nelson & Labuza, 1994; Blandamer *et al.*, 2005).

Water can directly participate in a reaction, as a reactant such as in a hydrolysis reaction or as a product, such as in a condensation reaction. On the other hand, water activity can influence the rate of a reaction by affecting the concentration of compounds involved in the reaction pathway, and/or their thermodynamic activity (Van Boekel, 2009). Water, finally, can act as a plasticizer and play a role in the rate of a reaction by affecting the molecular mobility in dense matrices.

In this study, the combined effect of temperature and water content (WC)/water activity (a_w) on GLs degradation in broccoli (*Brassica oleracea* var. *italica*) was investigated. A batch of broccoli was blanched by microwaving to inactivate the myrosinase, in order to rule out the influence of enzymatic breakdown (Verkerk & Dekker, 2004), and to focus entirely on thermal degradation of GLs. Broccoli was subsequently freeze dried to obtain batches with different WC/a_w. These batches of broccoli were then heated at different time/temperature combinations. To our knowledge this is the first time that GLs thermal degradation is studied and kinetically modelled in broccoli, including the effect of water content on GLs thermal degradation.

Materials and Methods

Materials

One batch of fresh broccoli (*Brassica Oleracea* var. *italica)* was purchased from a local supermarket (Company of the fruit, Wageningen, The Netherlands).

Sample Preparation

The stem was removed from the broccoli and the florets were cut into smaller pieces (size approximately 2 x 2 x 2 cm). A sample of fresh broccoli was analysed for GLs content, whereas the remaining part was microwaved in order to inactivate myrosinase as described by Verkerk (2002). The chopped broccoli florets were divided into portions of 300g, placed in a 1000 mL glass beaker and heated in a microwave oven at 900 W for 5 min (DAEWOO, Model KOC-87-T Korea). After microwave treatment the samples were cooled on ice and stored at -20°C over night. The frozen broccoli was then placed in aluminium trays and freeze dried for

times ranging from 1 to 7 days (GRInstruments, Model GRI 20-85 MP 1996, The Netherlands).

The trays were weighed day by day to check roughly the loss of WC in the samples. At the end five sub-batches of broccoli with different WC/a_w were selected to be used for the heating process. Those values were selected as representative of a wide range of WC/a_w.

Then the samples were frozen in liquid nitrogen and ground to a fine powder by using a Waring blender (model 34BL99, Dynamics Corp. of America, New Hartford, CT, USA). The samples were stored in a sealed container at -20 °C before they were used for the thermal treatment experiments. The WC of fresh, microwaved and microwaved/freeze dried samples was measured by gravimetric analysis. An aliquot of sample was placed overnight in an oven at 100°C, then cooled in a desiccator for 1 hour and finally weighed. The water activity of all samples was measured at 25°C using a LabMaster-a_w (Novasina Lachen, Switzerland).

Myrosinase Activity Determination

Fresh and microwaved broccoli (the amount was calculated to reach the same amount of dry matter as contained in 4 g of fresh broccoli) were mixed with 4 mL Milli-Q water, vortexed and centrifuged (Heraeus Multifuge X3R, DJB Labcare Ltd, England) at 4700 rpm, 4 °C, for 15 min to remove insoluble cell compounds. The supernatant was centrifuged again at the same conditions. In order to avoid the interference of the glucose naturally occurring in the broccoli sample, the supernatant from the latest centrifugation, was centrifuged at 25000 rpm 10°C for 30 min (Beckman High Speed Centrifuge Avanti J-26-XP, rotor JA 25-15). The resulting pellet was dissolved in 4 ml of ice cold phosphate (mixture of potassium dihydrogen phosphate and di-potassium hydrogen phosphate (Merck), 50 mM, pH 7.0), and the myrosinase was precipitated again under the same conditions. The obtained pellet was dissolved in 0.5 ml of ice cold phosphate buffer. The myrosinase activity was determined by following the hydrolysis of sinigrin by measuring the released glucose by a coupled enzymatic reaction.

The myrosinase activity was determined according to a coupled enzymatic procedure as described by Van Eylen *et al.* (2006), in which the produced glucose from the reaction of native myrosinase and a standard GL, was measured.

Thermal Breakdown

Frozen broccoli powder (1.5 g) was transferred into metal tubes (diameter 1.5 cm, length 11.5 cm) with hermetic screw caps (to allow pressure build up at T>100 °C and to avoid water loss) and they were kept on ice until heating. A thermocouple was placed in one tube, through a septum in the cap, to monitor the temperature profile of broccoli powder during the heat treatment. The time-temperature combinations used are reported in Table 3.1. All the heating experiments were carried out in triplicate except for samples with 68% WC which were performed in duplicate, due to lack of available material.

Table 3.1: Heating times (min) and temperatures for broccoli with different water contents.

Water content %	60°C	80°C	100°C	120°C
13	360	36	5	1
	720	72	15	5
	1080	108	30	15
	1800	144	45	20
	2700	180	60	30
34	360	36	5	1
	720	72	15	3
	1080	108	30	10
	1800	144	45	18
	2700	180	60	30
56	360	120	-	2
	720	270	15	8
	1080	390	30	13
	1800	600	45	18
	2700	840	60	33
68	360	120	15	5
	720	270	60	15
	1080	390	80	20
	1800	510	120	30
	2700	720	150	-
82	360	120	15	5
	720	270	30	10
	1080	390	60	15
	1800	510	100	20
	2700	720	150	30

The samples were heated in a heating block (Labyrinth Holland BV Kerkdriel NR 200406900). The heating-up-time was between 2 and 3 minutes (irrespective of the WC of samples). This time was excluded from the kinetic parameter analysis. After heating, the samples were cooled on ice and analysed for glucosinolates content.

Glucosinolates Extraction and Analysis

The method described by Verkerk *et al.* (2001), based on extraction of glucosinolates (GLs) with hot methanol as solvent was used with minor modifications. Different amounts of dried broccoli were weighed according to the WC of the sample. This amount was calculated to reach the same amount of dry matter as contained in 1 g of fresh broccoli.

The samples were transferred into plastic tubes and an amount of water for each sample was added to obtain a final weight of 1 g, in order to reach the same WC of fresh sample (not microwaved and not freeze dried). Then 2.4 ml hot methanol (100%) and 200 µL of 3 mM glucotropaeolin solution (internal standard) were added. Samples were incubated in a water bath of 75°C for 25 min, and mixed every 5 min. After incubation, the samples were centrifuged for 10 min at 2500 rpm. The supernatant was collected in new 15 ml tubes. The pellet was re-extracted twice with 2 mL of hot methanol (70%), centrifuged and the supernatants were combined with the first supernatant.

The extracted GLs were desulphated and then analysed according to Oerlemans *et al.* (2006).

The desulfoglucosinolates were separated using a GraceSmart RP C18 column (150 mm x 4.6 mm) with a flow rate of 1 ml/min, an injection volume as 20 µl. Elution of desulfoglucosinolates from the HPLC column was performed by a gradient of water containing 0.05% tetramethylammoniumChoride (A) and acetonitril/milli-Q water (40:60, v/v) containing 0.05% tetramethylammoniumchoride (B).

The total running time was 31 min with a gradient elution as follows: 100% A and 0% B for 1 min, then in 20 min to 0% A and 100% B, and in 5 min back to 100% A and 0% B. A UV detector was used at a wavelength of 229 nm. Individual GLs were identified by comparison of the retention times and spectra of two standard GLs, sinigrin (2-propenylglucosinolate) and glucotropaeolin (benzylglucosinolate), and by comparison of known GLs present in reference Brassica samples.

The quantification was carried out by using glucotropaeolin as internal standard and the relative response factor of each specific GLs. If peak areas were below the detection limit the samples were not used for data analysis and modelling.

Statistics and Modelling

Significance testing was done by performing an Anova test for the difference in GLs content among microwaved/freeze dryed broccoli and Student's *t* test for the difference in GLs content between microwaved broccoli and raw broccoli and for the differences between the reaction rate constants of aliphatic and indolic GLs for each temperature and each WC (rate constants of glucoraphanin and glucoiberin compared to those of glucobrassicin, 4-methoxyglucobrassicin and neoglucobrassicin). Both the statistical analyses were performed in Microsoft Excel 2007.

Thermal degradation of GLs was described with a first-order reaction model (Equation 3.1). Reaction kinetics modelling and parameter estimations were carried out by global fitting of the data sets. Global fitting implies that the models were fitted to the data sets for each GL in the same WC/a_w sample at all time/temperature incubations simultaneously to estimate the kinetic degradation parameters. The software package Athena Visual Workbench (www.athenavisual.com) was used for numerical integration of differential equations as well as parameter estimation of the reaction kinetic parameters (reaction rate constant $k_{d,i,ref}$, min^{-1}, and activation energy E_a kJ/mol).

$$\frac{d[GL_i]}{dt} = -k_{d,i}[GL_i] \qquad \textbf{Equation 3.1}$$

The concentrations of GL were expressed as µmol/g FW. For the parameter estimation all the individual measured concentrations were used from the triplicate experiments (except the samples with 68% WC, performed in duplicate), thus taking into account all the variability in the samples. Temperature dependence of the reaction rate constants (k_d) was described by the rearranged Arrhenius equation:

$$k_{d,i} = k_{d,i,ref} \exp\left\{\left(\frac{E_{a,i}}{R}\right)\left(\frac{1}{T_{ref}} - \frac{1}{T}\right)\right\} \qquad \textbf{Equation 3.2}$$

As a result the reaction rate constants were no longer expressed as a function of the pre-exponential factor, but as a function of a reaction rate constant relative to a reference temperature and the activation energy. The temperature of 100° C was used as the reference temperature (T_{ref} = 373 K). Estimation of reference reaction rate ($k_{d,i,ref}$) and Activation Energy ($E_{a,i}$) of each GL was achieved by global fitting of Eqs. (3.1) and (3.2) simultaneously to the experimental data of all times and temperatures investigated for each GL separately. The initial concentrations were estimated by the fitting procedure as well, to allow for uncertainty in the experimental observation at t=0. T is the sample temperature (K) and R the universal gas constant (8.32 J/mol K).

Result and discussion

Identified Glucosinolates

The identified GLs in the present study were: glucoiberin (3-methylsulfinyl-propylglucosinolate), glucoraphanin (4-methylsulfinylbutylglucosinolate), glucobrassicin (3-indolylmethylglucosinolate), 4-methoxyglucobrassicin (4-methoxy-3-indolylmethylglucosinolate) and neoglucobrassicin (N-methoxy-3-indolylmethyl-glucosinolate). The first two glucosinolates (glucoiberin and glucoraphanin) are classified as aliphatic glucosinolates, whereas the last three are classified as indolic glucosinolates.

Glucoraphanin was the most abundant GL, and hence the most abundant aliphatic GL, whereas neoglucobrassicin was the most abundant indolic GL in this batch of broccoli. Many studies reported glucoraphanin as the predominant GL in broccoli. However, variable GLs profiles are reported in literature in broccoli (Kushad *et al.*, 1999; West *et al.*, 2002; Tian *et al.*, 2005).

Glucosinolates and myrosinase after microwaving and water content/water activity and glucosinolates after freeze drying

To discriminate the effect of thermal breakdown of GLs from that of enzymatic breakdown, the endogenous myrosinase was first inactivated by blanching with a microwave treatment. After the microwave treatment, myrosinase activity was not detectable in broccoli. The GLs content of the broccoli increased slightly after the

microwave treatment (see Table 3.2) but only the content of 4-methoxyglucobrassicin and neoglucobrassicin was found to be significantly different ($P<0.05$) compared to fresh broccoli. This slight increase could be due to an induced extractability of GLs after mild heat treatment, as it was reported by Verkerk *et al.* (2004) who observed a 78% increase of total GLs content in red cabbage after microwave treatment at similar conditions (300g at 900W for 4.8 min). A decreased GLs content in fresh samples, due to myrosinase activity, can be excluded, because samples were always kept frozen in liquid nitrogen, till the GLs extraction.

Table 3.2: Comparison between glucosinolates content (± SD) in fresh and microwaved broccoli (µmol/g FW). For each GL, different letters refer to statistically different values ($P<0.05$).

Glucosinolates	Fresh Broccoli	Microwaved Broccoli
Glucoraphanin	0.80 ± 0.07[a]	0.85 ± 0.06[a]
Glucoiberin	0.32 ± 0.03[a]	0.36 ± 0.03[a]
Glucobrassicin	0.09 ± 0.01[a]	0.10 ± 0.01[a]
4-methoxyglucobrassicin	0.014 ± 0.001[a]	0.031 ± 0.001[b]
Neoglucobrassicin	0.16 ± 0.01[a]	0.20 ± 0.01[b]

Subsequently microwaved broccoli was freeze dried to obtain batches of broccoli with different WC and a_w (see Table 3.3).

Figure 3.1 shows the GLs content in microwaved and subsequently freeze dried broccoli. After correcting for the difference in dry matter content of the samples no significant difference for each GL were found among the samples with different WC ($p>0.05$).

Table 3.3: Water content and water activity values (± SD) of 5 batches of freeze dried broccoli.

	Sub-Batch 1	Sub-Batch 2	Sub-Batch3	Sub-Batch 4	Sub-Batch 5
Water content (%)	13.0 ± 0.7	34.0 ± 0.1	56.0 ± 0.2	68.0 ± 0.1	82.0 ± 0.1
Water activity (at 25° C)	0.32 ± 0.02	0.83 ± 0.03	0.94 ± 0.05	0.96 ± 0.02	0.99 ± 0.01

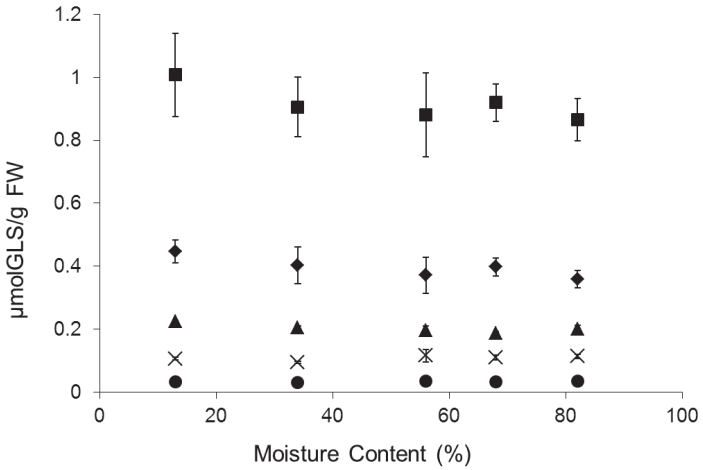


Figure 3.1: Glucosinolates content in broccoli after freeze drying to different water contents (■ glucoraphanin, ♦ glucoiberin, ✖ glucobrassicin, ● 4-methoxyglucobrassicin and ▲ neoglucobrassicin) in microwaved and freeze dryed broccoli (13, 34, 56, 68, and 82%). The concentration is recalculated to the original FW of the fresh broccoli for easier comparison.

Thermal degradation and modelling

Batches of broccoli with different WC were heated at temperatures ranging from 60 to 120°C for various times (Table 3.1), in order to investigate the influence of the WC/ a_w on the rate of GLs thermal degradation. All the experimental data derived from the thermal treatments were used simultaneously to determine the kinetic parameters ($k_{d,i',100°C}$ and $E_{a,i}$) using Eqs. (3.1) and (3.2). The kinetic parameters and their SD for the degradation of glucosinolates are summarized in Table 3.4 and 3.5. The GLs degradation was well described (judged by analysis of the residuals) by first order reaction kinetics and the temperature dependence of the reaction rate constants could be described by the rearranged Arrhenius equation. Figure 3.2 and 3.3 show the thermal degradation of the main aliphatic and the main indolic GLs in broccoli, glucoraphanin and neoglucobrassicin, as a function of time for the samples with 13, 34 and 82% WC.

Table 3.4: First-order thermal degradation parameters for broccoli with different water content: reaction rate constant at 100°C.

Glucosinolates	$k_{d,100°C}$ ± SD (×10^{-2} min^{-1})				
	Water Content (%)				
	13	34	56	68	82
Glucoraphanin	2.70 ± 0.30	6.70 ± 0.44	4.10 ± 0.32	0.90 ± 0.22	1.50 ± 0.09
Glucoiberin	1.90 ± 0.16	6.50 ± 0.43	3.80 ± 0.28	2.3 ± 0.16	1.50 ± 0.12
Glucobrassicin	1.70 ± 0.13	3.40 ± 0.44	2.60 ± 0.30	2.40 ± 0.16	1.30 ± 0.09
4methoxyglucobrassicin	2.60 ± 0.26	7.90 ± 0.74	3.80 ± 0.23	4.90 ± 0.26	2.40 ± 0.19
Neoglucobrassicin	1.30 ± 0.08	4.40 ± 0.44	3.10 ± 0.18	2.60 ± 0.16	1.30 ± 0.08

As a general observation, regardless the WC of the samples, increasing the temperature of the treatments resulted in an increase of the degradation rate for each GL. Similar results were obtained by Oerlemans *et al.* (2006) when heating red cabbage after myrosinase inactivation and by Dekker *et al.* (2009) when heating five different *Brassica* vegetables, including broccoli.

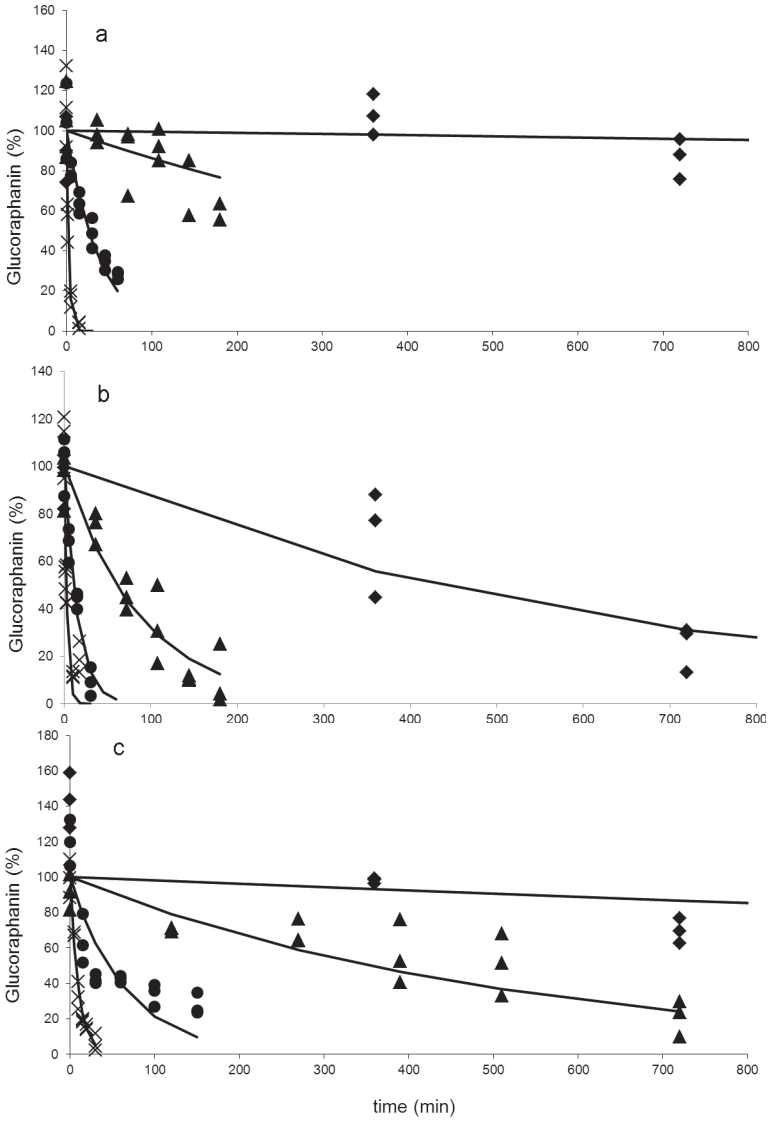


Figure 3.2: Thermal degradation of glucoraphanin in broccoli with different water content and a_w: **a** (13%, 0.32); **b**.(34%, 0.83); **c**(82%, 0.98). Thermal treatment: 60 (♦); 80 (▲);100 (●); 120°C (✖). The lines represent the fitted degradation profiles by the model.

In our study, no significant differences (p>0.1) were observed between aliphatic and indolic GLs degradation rate constants (k_d values of glucoraphanin and glucoiberin compared to k_d values of glucobrassicin, 4-methoxyglucobrassicin and neoglucobrassicin) for each temperature and each WC tested.

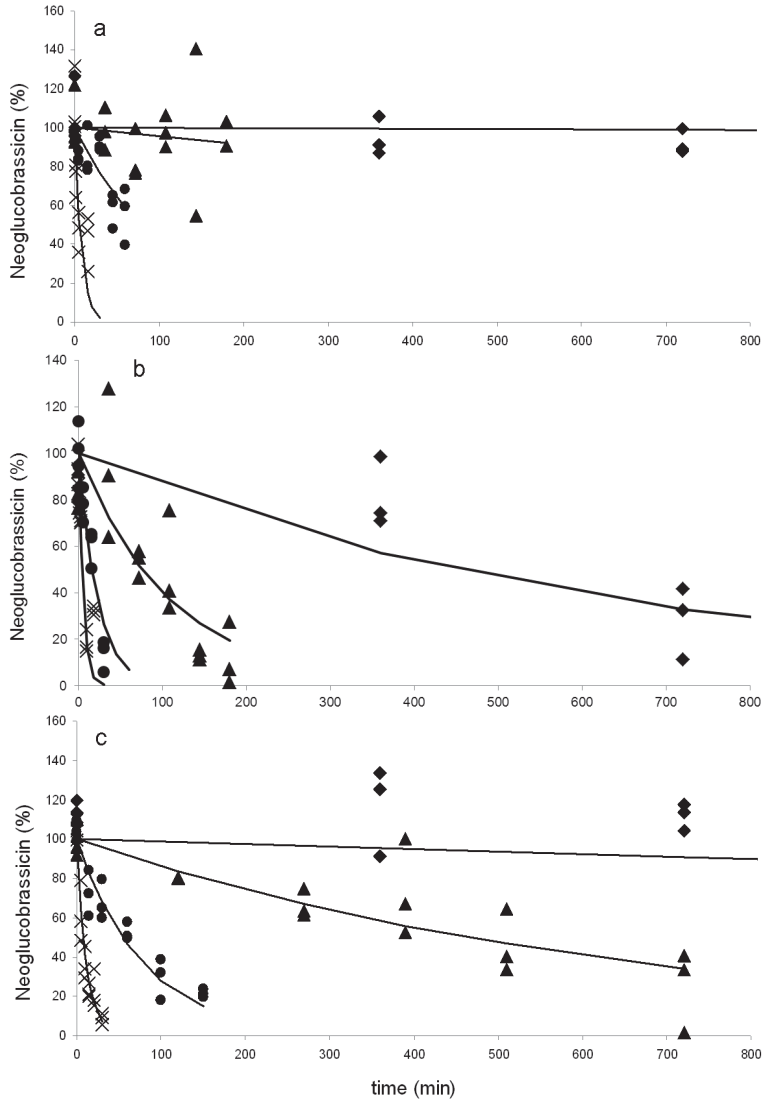


Figure 3.3: Thermal degradation of neoglucobrassicin in broccoli with different water content and a_w: a (13%, 0.32); b.(34%, 0.83); c(82%, 0.98). Thermal treatment: 60 (♦); 80 (▲);100 (●); 120°C (✖). The lines represent the fitted degradation profiles by the model.

This is in contrast with the results reported by Oerlemans *et al.* (2006) who observed a different aliphatic and indolic GLs thermal degradation rates in red cabbage: the indolic GLs showed higher degradation rate constants at lower temperature (<110°C), whereas at higher temperature (>110°C) the degradation rate constants of indolic GLs are comparable with the degradation rate constants of the aliphatic ones.

In our study we noticed that 4-methoxyglucobrassicin showed the highest degradation rate constants among indolic GLs, whereas concerning the aliphatic ones, glucoraphanin and glucoiberin showed similar rate constants in most of the samples investigated.

Dekker *et al.* (2009) reported the same order of glucosinolates thermal degradation rate constants for all five investigated vegetables: 4-methoxyglucobrassicin > glucobrassicin > gluconapin.

Big differences between the degradation rate constants of all GLs in samples with different WC were noticed (Figure 3.4).

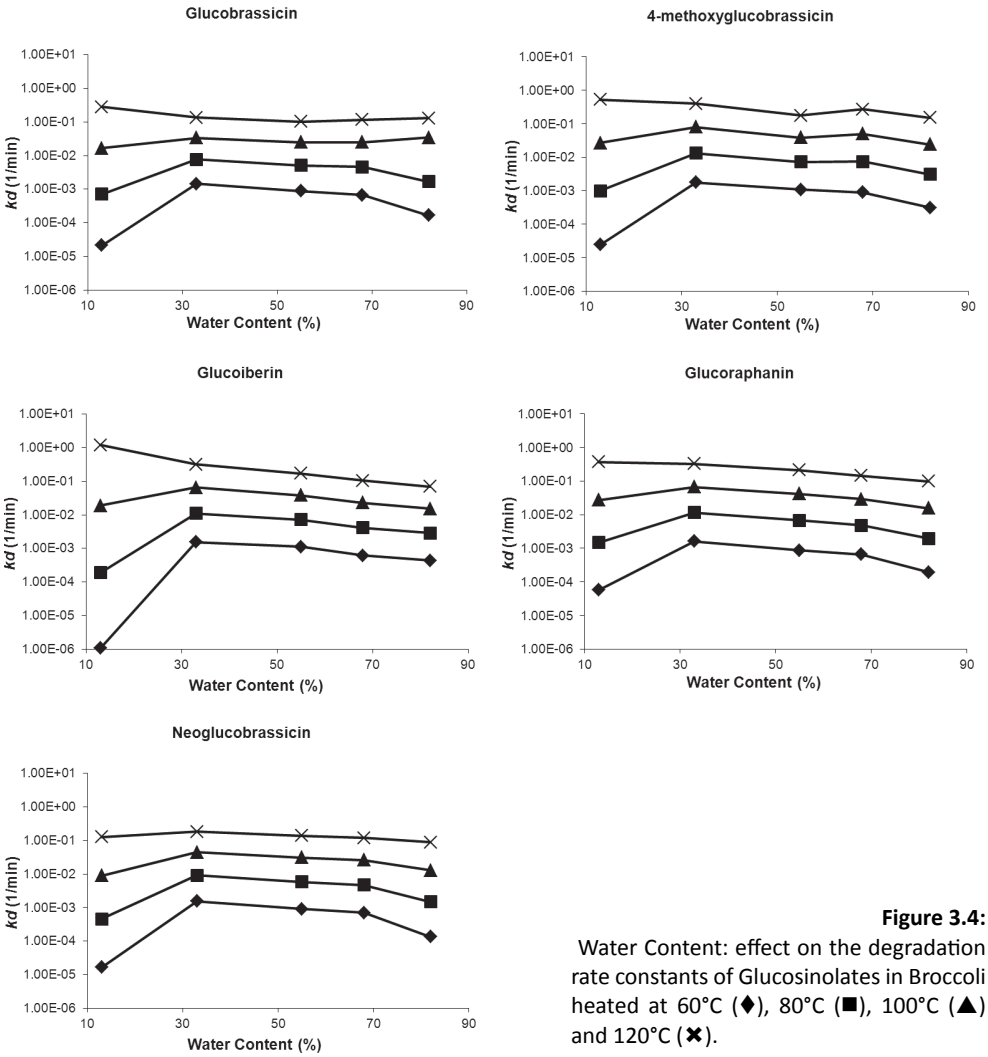


Figure 3.4:
Water Content: effect on the degradation rate constants of Glucosinolates in Broccoli heated at 60°C (♦), 80°C (■), 100°C (▲) and 120°C (✖).

The degradation rate constants increased with the WC in the samples with 34, 56, 68 and 82% WC for each GL, regardless of the temperatures used. On the contrary, the samples with 13% WC showed the lowest degradation rate constants at 60, 80 and 100°C for all GLs. Interestingly at 120°C the GLs degradation rate constants in the samples with 13% became the highest for all GLs except for neoglucobrassicin where the degradation rate constants were similar among the samples with different WC.

These results are reflected in the values of Activation Energy ($E_{a,i}$) reported in Table 3.5, in which the highest values of $E_{a,i}$ were found in the broccoli samples with 13% WC. Since the E_a value represents the temperature-dependency of the rate constants, the rate constants of the samples with 13% WC were the most affected by the increase in temperature. Moreover, as reported in Table 3.5, a general trend towards higher values of E_a as the WC increases from 34 to 82% can be noticed for glucoraphanin, glucobrassicin, 4-methoxyglucobrassicin and neoglucobrassicin whereas this does not hold for glucoiberin, which values of E_a were similar in the samples with WC between 34 and 82%.

Most of the values of the $k_{d,i}$ and of the $E_{a,i}$ that are reported in this study are similar to those reported by Oerlemans *et al.* (2006) for GLs degradation in red cabbage. However, some notable difference can be found as well. For instance, the E_a value of glucoiberin (92 ± 3 kJ/mol) in the samples with 82% WC was considerably lower than that reported for non-dried red cabbage by Oerlemans *et al.* (2006) (203 ± 7 kJ/mol). Also the E_a value of glucoraphanin and glucobrassicin in partly dried broccoli were lower, whereas the E_a of 4-methoxyglucobrassicin was similar when compared to non-dried red cabbage. These differences could be explained taking into account the different WCs of broccoli samples investigated in this study and those of red cabbage investigated by Oerlemans *et al.* (2006). These two vegetables, even though they belong to the same vegetal family, exhibit a different structure so the matrix where the glucosinolates degradation took place was different, thus resulting in a different kinetic behaviour. A strong effect of the plant matrix on GLs thermal degradation rates was also shown by Dekker *et al.* (2009).

Table 3.5: First-order thermal degradation parameters for broccoli with different water content: Activation Energy.

Glucosinolates	E_a (kJ/mol) ± SD				
	Water Content (%)				
	13	34	56	68	82
Glucoraphanin	159 ± 7	96 ± 5	99 ± 3	98 ± 3	113 ± 2.5
Glucoiberin	253 ± 10	97 ± 3	91 ± 3	93 ± 3	92 ± 3
Glucobrassicin	170 ± 7	82 ± 5	86 ± 5	93 ± 2	110 ± 3
4methoxyglucobrassicin	180 ± 10	98 ± 3	92 ± 2	104 ±2	110 ± 3
Neoglucobrassicin	162 ± 12	86 ± 4	91 ± 2	93 ± 2	120 ± 3

To explain the observed phenomenon of the degradation rates for each GL as a function of the WC/a_w we will discuss several hypotheses. A first hypothesis is that the heat transfer during the incubations is affected by the WC of the samples. However, the samples showed almost identical time-temperature profiles during the heating process, irrespective of the WC of the samples. For this reason the hypothesis that the observed behaviour could be due to different thermal properties of the samples or to the occurrence of evaporative cooling interfering with the time-temperature profiles inside the samples can be discarded.

In the following discussion we will assume that GLs degradation is governed solely by a hydrolysis reaction. Indeed, at the best of our knowledge, no GLs degradation mechanism alternative to hydrolysis has been reported so far. In addition, the thermal dependence of the rate constants was very well described by the Arrenhius equation. Although this does not represent a compelling evidence for the hypothesis that the thermal degradation was governed by a one step reaction with a single energy barrier as a hydrolysis reaction, since even complex reaction may exhibit the same dependence. But it does not even disprove such hypothesis. Under this assumption, the behaviour exhibited at the highest temperature studied, *i.e.* the highest k_d constants in the driest broccoli, might be explained by the effect on the thermodynamic activity of reactants and of the activated complex. According to the transition state theory, the rate of a reaction in non-ideal solution (such as dried

broccoli) depends on the activity coefficients of reactants and that of the activated complex. All the factors able to modify the activities of reactants and of the activated complex have an impact on the rate constant of the reaction (Van Boekel, 2009). In non-ideal conditions the following relation holds for a bimolecular reaction:

$$k_{obs} = k_{id}\left(\frac{\gamma_{GL} * \gamma_{H2O}}{\gamma \ddagger}\right) \qquad \textbf{Equation 3.3}$$

Where k_{obs} is the observed rate, k_{id} is the kinetic rate in ideal conditions and γ_{GL},γ_{H2O} and $\gamma_{\ddagger}$ are the activity coefficients for GL, H_2O and the transition state respectively. Should the activity coefficients change in a way that the ratio (γ_{GL}*γ_{H2O}/$\gamma_{\ddagger}$) increases, the rate of the reaction would increase. Even though we cannot predict the change in the activity coefficient of the activated complex for the hydrolysis reaction of GLs, nevertheless the activity coefficients of GLs are expected to increase as the a_w decreases. Were the increase in the activity coefficient of GLs to be strong enough and such that the ratio (γ_{GL}*γ_{H2O}/$\gamma_{\ddagger}$) to be >1, the rate of the hydrolysis reaction would increase despite the decrease of the activity coefficient of water. This hypothesis would explain the order of degradation rates of GLs as observed in the samples between 34 and 82% WC, but hardly could explain the lowest degradation rate of the sample with 13% WC at low temperatures. An explanation for this could be that in such a sample the very low a_w would impact the molecular mobility of the reactants. The GLs hydrolysis reaction would switch from reaction-limited to diffusion-limited in the conditions as occurring in the sample with 13% WC (Van Boekel, 2009). At higher temperatures, as the molecular mobility increases, the reaction rate would be no longer (or less) diffusion-limited and the rate constants would exceed those of the wetter samples. In addition, it is well known that, at constant water content, the a_w increases with temperature and that the relative increase in a_w is inversely related to the initial water content of the food sample (Van Boekel, 2009). For that reason, when heated at 100°C and 120°C, the sharper increase in the thermodynamic activity of water and in the molecular mobility of the reactants in the driest samples might contribute to explain the observed results. The change of a_w at 120 ˚C, as expressed by the Clausius-Clapeyron equation, was quantified. By calculating the heat of sorption of the sample with 13% WC from the sorption isotherm of broccoli (Jin *et al.,* 2013), the a_w at 120 ˚C was 0.47 (at 25 ˚C

it was 0.32). Hence, the a_w of the driest samples slightly increased at 120 ˚C, but it did not reach the a_w of the higher water content broccoli (Table 3.3). It is likely that both these hypothesis, namely effect of a_w on activities and mobility of reactants contribute to the effect of WC on the degradation rates of GLs in dried broccoli.

Conclusion

Glucosinolate degradation rate constants increased with temperature for all WC studied (13% to 82%). No significant difference in degradation rate constants was found between aliphatic and indolic glucosinolates. The most remarkable outcome of this study was the influence of WC on the glucosinolate degradation rate constants: at temperatures of 100°C and lower the sample with 13% WC shows the lowest glucosinolate thermal degradation rate constants whereas an increase in degradation rate constants was observed as the WC decreased (from 34 up to 82%). At 120°C the order of degradation rate constants changed in such a way that GLs in the driest sample now showed the highest degradation rate constant. This behaviour is reflected by the much larger E_a of the degradation reaction in the driest sample compared to all other samples. For that reason, the difference in degradation rate constants for the different WC's is markedly reduced as the temperature increases. This particular effect of the WC on the degradation rates might be explained by two mechanisms: 1) the indirect effect of the water content on the thermodynamic activity of water, glucosinolates and the activated complex of the hydrolysis reaction and 2) the effect of the WC on the molecular mobility of the reactants. Further research is needed to elucidate the role of water content on the thermodynamic activities of the glucosinolates and its effect on molecular mobility of the reactants. Further research can also be done on the occurrence of other reactions during the thermal treatment of non-blanched broccoli focusing not only on the glucosinolates degradation, but also on the changes in the myrosinase activity (which was excluded in the present study) and the formation of the enzymatic and non-enzymatic breakdown products. The results reported in this study are of importance because they prove that the water content/water activity of broccoli has a clear influence on the rate of glucosinolates thermal degradation. These findings are important for the optimization of the process conditions during hot air drying in order to obtain dried broccoli which retains as much as possible of the glucosinolates content of fresh broccoli.

Acknowledgements

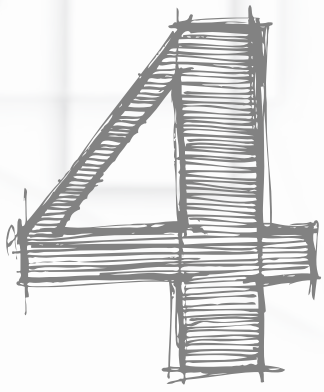
This work is supported by the Energy Research Program EOS of the Dutch ministry of Economics under project EOSLTe7043. The authors thank Prof. Dr. Ir. Tiny van Boekel for critical reading of the manuscript.

Effect of water content and temperature on inactivation kinetics of myrosinase in broccoli (*Brassica oleracea* var. *italica*)

Oliviero T.
Verkerk R.
Van Boekel M.A.J.S.
Dekker M.

Submitted for publication

CHAPTER



Abstract

Broccoli belongs to the *Brassicaceae* plant family consisting of widely eaten vegetables containing high concentrations of glucosinolates. Enzymatic hydrolysis of glucosinolates by endogenous myrosinase (MYR) can form isothiocyanates with health-promoting activities. The effect of water content (WC) and temperature on MYR inactivation in broccoli was investigated. Broccoli was freeze dried obtaining batches with WC between 10% and 90% (a_w from 0.10 to 0.96). These samples were incubated for various times at different temperatures (40-70 ˚C) and MYR activity was measured. MYR inactivation was best described by a consecutive step reaction model and inactivation rate constants and activation energies were estimated for all WC samples. MYR inactivation rate constants were lower in the driest samples (10% WC) at all studied temperatures. Samples with 31%, 67% and 90% WC showed inactivation rate constants all in the same order of magnitude. These results are useful to optimize drying processes to produce dried broccoli with optimal MYR retention for human health.

Introduction

Myrosinase (MYR) is a thioglucoside glucohydrolase (EC 3.2.3.147) that occurs in all glucosinolates-containing plants, such as Brassica vegetables. MYR can consist of multiple isoforms that differ in molecular weight due to different degrees of glycosylation (Bones & Rossiter, 1996). Glucosinolates are a group of secondary plant metabolites that are physically separated from MYR in intact plant tissue (Verkerk *et al.*, 2009). Cellular disruption caused by, *e.g.*, insects, food processing, preparation and mastication, enables MYR to catalyze the hydrolysis of glucosinolates. The hydrolysis products are unstable compounds that spontaneously break down into a range of bioactive compounds including isothiocyanates (Kissen *et al.*, 2009). In recent years, isothiocyanates received a lot of attention because of their potential anticarcinogenic effect against several types of cancer (Verhoeven *et al.*, 1997). For optimization of health promoting properties of *Brassica* vegetables, it is important that the beneficial compounds are retained during food processing and storage. When MYR activity is reduced, the isothiocyanate formation, hence their health promoting effect, is diminished (Shapiro *et al.*, 1998). Colonic bacteria also produce glucosidases that can hydrolyse glucosinolates, but the hydrolysis extent by colonic enzyme activity has been reported to be lesser compared to the action of plant MYR (Shapiro *et al.*, 1998). Therefore, being able to retain MYR activity during processing would results in a more healthy final product. Among the food processing, high temperature treatments lead to MYR inactivation (Verkerk *et al.,* 2009). For instance, upon domestic cooking MYR can be inactivated (Verkerk *et al.,* 2009). MYR activity in cabbage was effectively lost after 2 min of microwave cooking at 750 W or after 7 min of steaming (Rungapamestry *et al.*, 2006) or after microwave cooking for 4.8 min at 900 W (Verkerk & Dekker 2004).

The thermal inactivation kinetics of MYR have been studied in broccoli extract (Ludikhuyze *et al.*, 1999; Van Eylen *et al.*, 2006; Ghawi *et al.*, 2011), in vegetable juice (Van Eylen *et al.*, 2007) and intact broccoli (Van Eylen *et al.*, 2008).

No literature is available on MYR thermal inactivation in broccoli with different water contents (WC) and a_w. The a_w could affect the enzyme stability and thermal inactivation kinetics during heat treatments, *e.g.* by influencing the enzyme unfolding kinetics during heat treatment (Van Boekel, 2009). Such kinetic study

of MYR is very useful to optimize air drying process to obtain final products that retain glucosinolates and MYR. Drying of vegetables is applied to extend the shelf life of the vegetable products by reducing the a_w. Air drying technologies, which are widely used for preparation of ingredients for, *e.g.*, soups, sauces, apply relatively high temperatures that can have a significant impact on GLs content (Mrkic *et al.*, 2010) as well as on MYR activity.

In a previous study, performed by our group (Oliviero *et al.*, 2012 - **Chapter 3**), the influence of a_w on glucosinolate thermal degradation in broccoli was investigated. The determination of the kinetic parameters for the thermal stability of glucosinolates and MYR in broccoli with different a_w, is important for the optimization of their retention during air drying processes. These drying technologies, which are widely used for preparation of ingredients for, *e.g.*, soups, sauces, apply relatively high temperatures that can have a significant impact on MYR activity. Knowledge on the effect of a_w on the thermal stability of both glucosinolates and MYR can be used to optimize the drying process to retain maximum health benefit of the final vegetable product.

The aim of this study was to investigate the combined effect of temperature and WC/a_w of broccoli on MYR stability. For these purposes, broccoli was freeze dried to obtain batches with different WC/a_w. These batches of broccoli were then heated at different time/temperature combinations and MYR inactivation was analysed and kinetically modelled. The kinetic information obtained can be used to optimize drying processes for higher retention of active MYR.

Materials and Methods

One batch of fresh broccoli (*Brassica Oleracea* var. *italica*) was supplied by Fino Fresco (The Netherlands).

Sample Preparation

Stems were removed from broccoli and the bigger florets were cut into smaller pieces (size approximately 5 x 5 x 5 cm) and frozen at -20 °C overnight. Then, the frozen broccoli was placed in aluminium trays and freeze dried for times ranging from 1 to 7 days (GRInstruments, Model GRI 20-85 MP 1996, The Netherlands). The trays were weighed twice a day to check roughly the samples WC. Four sub-batches of broccoli with different WC/a_w were selected to be used for the heating process, including fresh broccoli with WC of 90%. Those values were selected as representative of a wide range of WC/a_w. Then, the samples were frozen in liquid nitrogen and ground to a fine frozen powder by using a Waring blender (model 34BL99, Dynamics Corp. of America, New Hartford, CT, USA). The samples were stored in a sealed container at -20 °C. The WC of the samples was measured by gravimetric analysis. For determining the WC an aliquot of sample was placed overnight in an oven at 100 °C, then cooled in a desiccator for 1 hour and finally weighed. The a_w of each sample was measured at 25 °C using a LabMaster-aw (Novasina Lachen, Switzerland).

Thermal Treatment

Frozen broccoli powder was transferred into metal tubes with caps that could be hermetically closed and kept on ice until heating. The samples were heated in a heating block for different times (Labyrinth Holland BV Kerkdriel NR 200406900). A thermocouple was placed in one tube, through a septum in the cap, to monitor the temperature profile of broccoli powder during the heat treatments. The incubation temperatures were 40, 50, 60 and 70 ˚C. All the heating experiments were carried out in duplicate. The heating-up-time was around 3 minutes (irrespective of the WC of samples). This timepoint was used in the kinetic parameter estimation as the starting time for the data analysis. After heating, the samples were cooled on ice and then the MYR activity was measured.

Myrosinase determination

The MYR activity was determined according to a coupled enzymatic procedure as described by Van Eylen *et al.* (2006), with some modification. To extract MYR from the broccoli matrix, samples (the amount was calculated to be equivalent to 0.05 g dry matter) were stirred using a stirring plate, at 15 ˚C in 140 mL of potassium phosphate buffer (50 mM pH 7.0) overnight. The day after, the MYR solutions were centrifuged at 2670 g for 10 min and the supernatants were filtered (folded filters Grade 595 ½- 4-7 µm, Whatman) to further clean the solution. Then, MYR was purified and concentrated by using filter tubes (Amicon Ultra-4 cut-off 30kD, Millipore) to remove the compounds with molecular weight lower than MYR (sugars, glucosinolates, etc.). The concentrated enzyme was dissolved again in 0.5 ml potassium phosphate buffer (50 mM pH 7.0). The MYR activity was determined according to a coupled enzymatic procedure as described by Van Eylen *et al.* (2006), with some modification. In this assay the D-glucose formed by the reaction between MYR and sinigrin as a substrate could be used to transform NADP+ to NADPH (D-Glucose kit, Enzyplus, Biocontrol). The reaction mixture consisted of 0.9 ml of a water solution containing 0.05 g/l $MgCl_2$ and 1 g/l ascorbic acid, 50 µl of the extracted MYR solution, 50 µl of test kit solution R1 (imidazole buffer, magnesium chloride and sodium azide), 50 µl of test solution R2 (NADP+, ATP), 5 µl of test solution R3 (hexokinase (300 U/ml), glucose-6-phosphate dehydrogenase (400 U/ml) and 50 µl of sinigrin solution (30 mg/ml). The formation of NADPH was followed by a spectrophotometer (Cary UV 50, Bergen op Zoom, The Netherlands) at 340 nm for 7 min. The activity was determined based on the slope of the linear part of the curve of absorbance versus reaction time. Activity was expressed as U/mg dry weight.

Statistics and Modelling

The kinetic modelling of MYR inactivation, the kinetic model was selected based on residual analysis and the Akaike criterion; Three models were tested: first order inactivation of a single enzyme, first order inactivation of two distinct isoenzymes, and the consecutive step reaction. The consecutive step model describes the MYR inactivation as a succession of two irreversible reaction steps with two distinct inactivation rate constants (Equation 4.1). In the first step the native enzyme structure changes to an intermediate form with lower activity than the native form.

In the second step, the intermediate form changes to the fully inactive form.

$$\mathrm{N} \xrightarrow{k_1} \mathrm{Y} \xrightarrow{k_2} \mathrm{I}$$

$$\frac{\mathrm{d}[\mathrm{N}]}{\mathrm{d}t} = -k_1[\mathrm{N}]$$

$$\frac{\mathrm{d}[\mathrm{Y}]}{\mathrm{d}t} = -k_2[\mathrm{Y}] + k_1[\mathrm{N}] \qquad \textbf{Equation 4.1}$$

$$\frac{\mathrm{d}[\mathrm{I}]}{\mathrm{d}t} = k_2[\mathrm{Y}]$$

N represents the native enzyme form, Y the intermediate enzyme form and I the inactive enzyme. k_1 is the inactivation rate constant of the first enzyme form (N) (min^{-1}) and k_2 is the inactivation rate constant of the second enzyme form (Y) (min^{-1}). The MYR activity was expressed as unit of MYR/g of FW. The temperature dependence of the k_i was described by the reparameterized Arrhenius equation:

$$k_i = k_{i,ref} \exp\left\{\left(\frac{E_{a,i}}{R}\right)\left(\frac{1}{T_{ref}} - \frac{1}{T}\right)\right\} \qquad \textbf{Equation 4.2}$$

T is the sample temperature (K) and R the universal gas constant (8.32 J/(mol K)). As a result the reaction rate constants were no longer expressed as a function of the pre-exponential factor, but as a function of a reaction rate constant relative to a reference temperature and the activation energy. As reference temperature, 50 °C was used (T_{ref} = 323 K). The reaction kinetics modelling and parameter estimations were carried out by global fitting of Eqs. (4.1) and (4.2) simultaneously at all time/temperature incubations investigated for each WC. The software package Athena Visual Workbench (www.athenavisual.com) was used for numerical integration of the differential equations as well as the parameter estimation of the reaction kinetic parameters (reaction rate constant $k_{i,ref,}$ min^{-1}, and activation energy E_a kJ/mol), the initial activity of N, the relative activity of Y (activity Y /activity N) and initial concentration of Y as fraction of N (Y=FY*N).

Significance testing was done by performing an ANOVA test for the difference in MYR activity among fresh and differently freeze dried broccoli (different WC broccoli). An ANOVA test was also performed to compare the influence of WC on k_1, k_2, E_1 and E_2 values.

Results and Discussions

Effect of water content/a_w on myrosinase activity

Four batches of broccoli varying in WC/a_w were analysed for their MYR activities (Table 4.1). The batches varied from fresh broccoli with 90% WC to almost complete dry broccoli with 10% WC. The WC values are related to the a_w values in a non-linear relationship that is the moisture isotherm curve. Similar relationship between WC and a_w was also found by Oliviero *et al.* (2012). The MYR activity in fresh broccoli used in this study was 59 ± 5 U/g DW. MYR activity can vary among different botanical groups and different growing seasons. For instance, MYR activity in the same broccoli cultivar (Emperor) grown during two autumn seasons and two spring seasons, was reported to vary between 151.7 U/g FW and 51.1 U/g FW (Charron *et al.*, 2005). Broccoli was freeze dried to remove water while preventing MYR inactivation. Indeed, no significant differences in MYR activity were found among fresh and freeze dried broccoli with different WC/a_w ($p > 0.05$) (Table 4.1).

Table 4.1: Water content (WC), water activity (a_w) and myrosinase activity (MYR) values (±SD) of 4 batches of freeze dried broccoli.

	Sub-batch 1	Sub-batch 2	Sub-batch 3	Sub-batch 4
WC (%)	90.0 ± 0.4^a	67.0 ± 0.7^b	31.0 ± 0.2^c	10.0 ± 0.6^d
a_w (at 25 ˚C)	0.96 ± 0.01^a	0.96 ± 0.01^a	0.82 ± 0.01^b	0.10 ± 0.01^b
MYR (U/g DW)	59 ± 8.0^a	57 ± 3.4^a	58 ± 1.2^a	61 ± 3.0^a

Thermal inactivation and modelling

Batches of ground broccoli with different WC/a_w were heated at 40, 50, 60 and 70 ˚C for various times to investigate the thermal inactivation of MYR at different WC/a_w. Three distinct kinetic models for MYR thermal inactivation were compared: first order, consecutive and two distinct isoenzyme models. The hypothesis of a reversible step in MYR inactivation after cooling was experimentally tested for the samples with 10 % WC. The sample with 10 % of WC was heated at 70 ˚C and then stored at room temperature for five weeks. The MYR was analysed right after heating and once a week during the storage. The samples with higher WC could not

be tested because during the storage the samples got spoiled and that interfered with MYR activity measurement. No significant change in MYR activity was observed during the storage. However, it was not possible to unambiguously conclude that no renaturation of MYR occurred in the first of the two consecutive steps in the small timescale between the thermal treatment and the measurements. Based on residual analysis and Akaike criterion, the first order model was rejected (Table 4.2).The consecutive model and the distinct isoenzyme model gave similar Akaike and R-Square values (Table 4.2). The distinct isoenzyme model gave unrealistic E_a values (up to 706 kJ/mol) because the reported range of E_a of typical food reaction is from 50 to 150 kJ/mol for chemical reactions (first and second order) and from 200 to 500 kJ/mol for protein denaturation (Van Boekel, 2009), therefore it was excluded. The consecutive step order model has been already used to describe MYR thermal inactivation in MYR extract (Ludikhuyze *et al.*,1999; Van Eylen *et al.*, 2006; Ghawi *et al.*, 2011), vegetable juice (Van Eylen *et al.*, 2007) and intact broccoli (Van Eylen *et al.*, 2008). This model describes an enzyme inactivation that consist of two irreversible reactions (Equation 4.1). In the first step the native enzyme structure changes to an intermediate form with lower activity than the native form. At an increase in temperature, proteins can partially unfold and then they may refold into new conformations. These conformations are different from the native enzyme conformations but they are kinetically or thermodynamically stable structures and enzymatically less active. In the second step, the intermediate form irreversibly changes to the inactive form. Each step is described by its own reaction rate constant (k) and activation energy (E_a) values. For each WC all the experimental data derived from the thermal treatments were used simultaneously to determine the kinetic parameters ($k_{1,50}$, $k_{2,50}$ and $E_{a1,}$ E_{a2}) using Equations (4.1) and (4.2). The temperature dependence of the reaction rate constants was described by the reparameterized Arrhenius equation (Equation 4.2).

Table 4.2: Akaike and R-Square values of first-order, two distinct isoenzyme and consecutive step reaction models for all the thermal inactivation of myrosinase in broccoli with different water contents (WC).

	Models											
	First-order				Two distinct isoenzyme				Consecutive step reaction			
WC (%)	10	31	67	90	10	31	67	90	10	31	67	90
Akaike	4.25	5.10	2.67	2.86	3.69	3.17	2.55	2.95	3.54	3.33	2.72	2.86
R-Square	0.83	0.07	0.90	0.94	0.91	0.87	0.92	0.94	0.93	0.87	0.91	0.94

As a general observation, regardless of the WC of the samples, increasing the temperature of the treatments resulted in an increase of the MYR inactivation rate (Figure 4.2). In addition, the thermal inactivation of MYR varied considerably for broccoli with different WC (Figure 4.1 and 4.2).

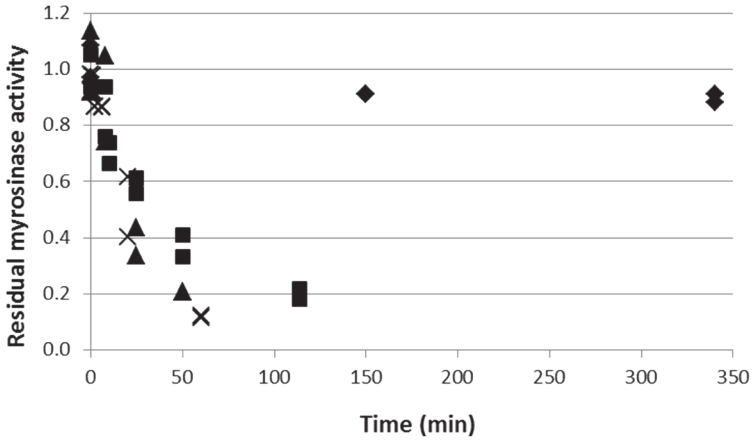


Figure 4.1: Thermal inactivation of myrosinase from broccoli with different water content at 50 ˚C. Water content: 10% (♦), 31% (■), 67% (▲) and 90% (✖).

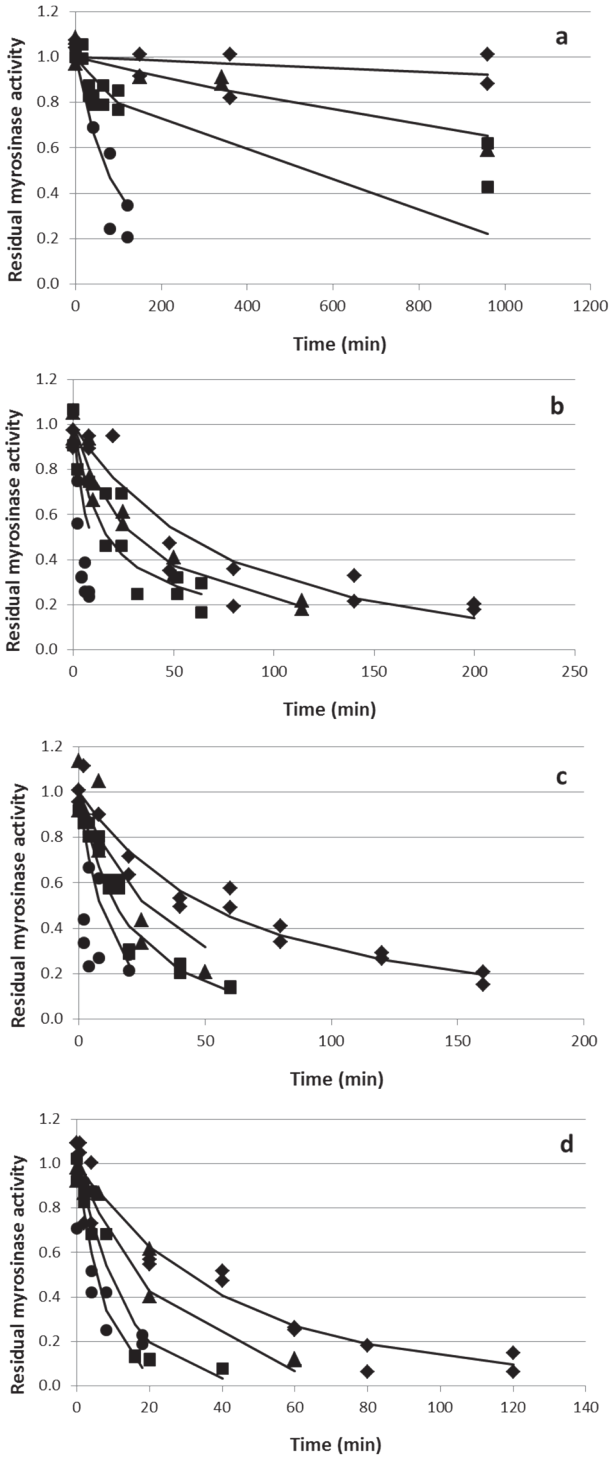


Figure 4.2: Thermal inactivation of myrosinase from broccoli with different water content: (**a**) 10 % (**b**) 31%; (**c**) 67 %; **d**) 90 %. Thermal treatments: 40 ˚C (♦); 50 ˚C (▲); 60 ˚C (■); 70 ˚C (●). The lines represent the fitted degradation profiles by the consecutive step model.

The estimated k_1 and k_2 of samples with 10% WC at all the heating temperatures were remarkably lower than the k_1 and k_2 of the other samples at all the temperatures (Table 4.3 and Figure 4.3). Furthermore, the k_2 was estimated as zero at 10% WC, suggesting that in the driest sample the second step reaction did not occur at the studied temperatures and times (Table 4.3 and Figure 4.3). Nevertheless, the statistical analysis (one-way ANOVA) did not reveal significant differences among the samples ($p>0.05$) which might depend on the limited number of replicates (n=2). The estimated k_1 and k_2 of samples with 90% WC at all the temperatures, were similar to the k_1 and k_2 values estimated by heating broccoli florets packed under vacuum (k_1 at temperature range of 50-60 ˚C, 6.4 ± 4.9 to 60.3 ± 9.0 (x 10^{-2} min^{-1}) and k_2 at temperature range of 50-60 ˚C, 1.1± 0.8-11.3 ± 2.8 x 10^{-2} min^{-1} (Van Eylen *et al.,* 2008). The residual enzymatic activity of the intermediate was estimated as a parameter in our model and is reported in Table 4.3. The residual activity of fresh broccoli (90% WC) was estimated at reference temperature (50˚C) as 25% and was considered constant at all the temperatures tested (Table 4.3). As a comparison, the residual MYR activities estimated by Van Eylen *et al.*(2008) heating fresh broccoli, were 48.6 ± 30.6 % at 50 ˚C and 25.6 ± 6.6 % at 60 ˚C.

4

Table 4.3. Consecutive step model: estimation of activation energy values (E_{a1} and E_{a2}), reaction rate constant at 50 ˚C ($k_{1,\,50\,^{\circ}C}$ and $k_{2,\,50\,^{\circ}C}$), the relative activity of the second enzyme form (Y) for thermal inactivation of endogenous MYR in broccoli.

Water Content (%)	Data point	$k_{1,\,50\,^{\circ}C}$ ± SD (x 10^{-2} min^{-1})	$k_{2,\,50\,^{\circ}C}$ ± SD (x 10^{-2} min^{-1})	E_{a1} ± SD (kJ/mol)	E_{a2} ± SD (kJ/mol)	Y± SD (%)
10	19	0.06 ± 0.02[a]	0.0	140 ± 18[a]	NE	16 ± NE
31	26	5.1 ± 1.1[a]	0.9 ± 0.3[a]	59 ± 20[b]	16 ± 29[a]	43 ± 19
67	27	4.8 ± 4.0[a]	1.2 ± 0.4[a]	51 ± 13[b]	62 ± 28[a]	37 ± 2
90	27	5.5 ± 1.2[a]	5.7 ± 8.5[a]	44 ± 16[b]	120 ± 124[a]	25 ± NE

NE = not estimated

Different letters within a column indicate significant differences ($p < 0.05$).

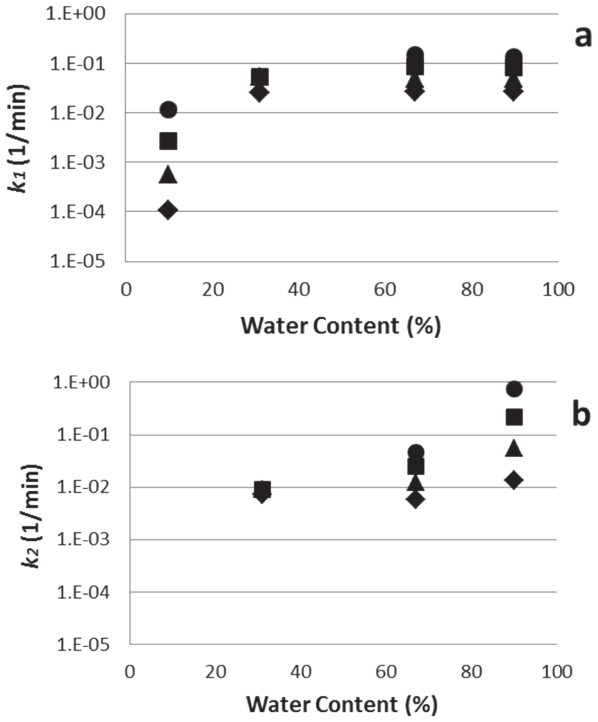


Figure 4.3: Consecutive step model: calculated inactivation rate constants of myrosinase in broccoli with different water content, for the first step reaction, k_1 (a) and the second step reaction, k_2 (b). Samples heated at 40 ˚C (♦); 50 ˚C (▲); 60 ˚C (■); 70 ˚C (●).The k_2 at 50 ˚C of broccoli with 10% of water content was estimated as zero.

The higher stability of MYR in dry broccoli samples may depend on many factors. Enzymes are known to be influenced by interactions with water, reflected in the a_w, in general, their stability increases with the decrease of a_w (Van Boekel, 2009). Thermal inactivation rate at 80 ˚C of lipoxygenase in a glucose calcium-alginate gel, described by the first order reaction, was lower at lower a_w (Liou, 1982). The thermal inactivation of peroxidase in horseradish was found lower in solid state than in solution (Hendrickx *et al.*, 1992). This phenomenon is linked to molecular crowding, which is basically a volume exclusion effect. In a molecularly crowded solution, the macromolecules are at a high concentration and occupy a large fraction of the volume, therefore less volume is available for a protein to unfold, compared to a dilute solution (Van Boekel, 2009). In such a crowded solution it becomes less favourable for enzymes to unfold, and this results in a remarkably higher thermal stability. In an environment with different water content the different interactions between the water molecules and the protein itself would produce enthalpic and entropic effects responsible for the different thermo stability. Another explanation to the different MYR heat stability in drier matrices may be a plasticizer effect of water.

Klibanov (1986) suggested that water acts as plasticizer, allowing conformational mobility of an enzyme which is necessary for a total or partial unfolding. MYR in the samples with 31%, 67% and 90% of WC will have a higher conformational mobility compared to samples with 10% WC.

The inactivation rate constants of the samples with 31- 90% of WC, had comparable inactivation rate constants differing substantially from the sample with 10% of WC. The a_w is very similar in those samples and identical for the sample with 67% and 90% of WC, whereas only the driest sample exhibited remarkable differences in the estimated k values and the a_w content.

The values of MYR inactivation energy (E_a) estimated by the model are reported in Table 4.3. To check whether the temperature dependency of rate constants k_1 and k_2 followed the Arrenhius law, the rate constants estimated by the consecutive model (k_1, k_2) at each single temperature, were plotted in an Arrenhius plot that showed a linear relationship between the log10 of each rate constant and the reciprocal of the absolute temperature (data not shown). It is interesting to notice that the E_a range of MYR is lower than the E_a range of general protein denaturation reported in literature, that is between 200-500 kJ/mol (Van Boekel, 2009). In fact the estimated MYR E_a falls into the range of chemical reactions (50-150 kJ/mol) (Van Boekel, 2009) that are less temperature–dependent than protein denaturation reactions. The E_a values estimated by Van Eylen *et al.* (2008) heating fresh broccoli were slightly higher (E_{a1} 202.4 ± 5.7 and E_{a2} 209.7 ± 5.8 kJ/mol) than our values, but they were in the lowest range for the E_a range of general protein denaturation reported in literature. The E_{a1} value for the driest broccoli (10% WC) sample was significantly higher than those for the other broccoli samples (one-way ANOVA, $p<0.05$). In the first reaction step of the consecutive model, the native enzyme structure changes to an intermediate form with lower activity than the native form. According to theoretical considerations, in a crowded environment the E_a of protein denaturation is higher than in a less crowded environment. Indeed when the transition state is more expanded than the reactant state which is the case in protein unfolding, molecular crowding is expected to increase the activation free energy and thus slow down the reaction rate (Zimmerman & Minton, 1993). Broccoli with 31% and 67% of WC showed similar E_a regardless of the steps reaction. The E_{a2} of the fresh broccoli (90%) was the highest, among second step reactions,

whereas the E_{a2} for the samples with 10% WC could not be estimated since the k_2 at 50 ˚C was estimated as zero. The effect of temperature on a_w as expressed by the Clausius-Clapeyron equation might also contribute to the higher E_a value in the first step for the driest sample. At the same WC, a_w increases with temperature. By calculating the heat of sorption (H_{st}, kJ/mol) of the sample with 10% WC from the sorption isotherm of broccoli (Jin *et al.,* 2013), the a_w at 70 ˚C was 0.13, thus slightly higher than at 25 ˚C (0.10). Hence, the a_w of the driest samples changed during heating, but the increase was negligible compared to the a_w differences between the different % WC samples (Table 4.1).

Conclusions

The aim of this study was to investigate the effect of temperature and WC/a_w on MYR inactivation in broccoli. The MYR inactivation was best described by the consecutive step model for each WC. The driest samples showed the highest MYR heat stability, whereas MYR stability in samples with 31%, 67% and 90% WC was comparable. The highest MYR stability in broccoli with 10% could be explained qualitatively by the molecular crowding/volume exclusion experienced by MYR and its conformational mobility.

The results obtained in this study, along with the results on the effect of WC and temperature on glucosinolates degradation (Oliviero *et al.*, 2012), are a useful tool to design drying processes to produce dried broccoli with optimal MYR and glucosinolate profiles, thereby optimizing its healthiness after processing.

Acknowledgements

This work is supported by the Energy Research Program EOS of the Dutch ministry of Economics under project EOSLTe7043.

Impact of different drying trajectories on degradation of nutritional components in Broccoli

Oliviero T.
Jin X.
Dekker M.
Van der Sman R.G.M.
Verkerk R.
van Boxtel A.J.B.

Submitted for publication

CHAPTER

Abstract

This work concerns the degradation of the nutritional components glucoraphanin (GR) and vitamin C (Vc), and the inactivation of the enzyme myrosinase (MYR) in broccoli during drying with air temperatures in the range of 30 to 60°C. Dynamic optimization is applied to find the optimized temperature trajectories that minimize degradation and inactivation. Simulation and experimental results for optimized temperature trajectories are compared to constant inlet air temperature drying at 40 and 50°C. The results show that with the optimized temperature trajectories the retentions of GR, MYR and Vc improved significantly. Moreover, the experiments show that degradation and inactivation upon drying is slower than expected from kinetic studies. The deviation is explained from the difference in the physical state of the samples used in the drying experiments, *i.e.* original plant tissue, and the grounded state of the plant tissue used in the experiments for the kinetic studies. The results indicate that besides temperature and moisture content the physical state is also an important aspect in the degradation of nutritional components and enzymes.

Introduction

Fruits and vegetables are rich in nutritional components such as vitamin C and folates, and phytochemicals as flavonoids, carotenoids, and glucosinolates. For instance, vitamin C is an essential nutrient, found widely in fruits and vegetables that can prevent diseases like scurvy and reduce the risk of heart disease and cancer (Wang & Stone 2008; Martin, 2012). Phytochemicals are chemical compounds that occur naturally in plants and contribute to the colour and other organoleptic properties of fruits and vegetables. Glucosinolates are a specific group of phytochemicals that occur in *Brassica* vegetables, such as broccoli, cauliflower, cabbage etc. They are studied for their protective effects against many types of cancer (Verhoeven *et al.*, 1997). Glucosinolates are a group of secondary plant metabolites (β-thioglycoside N-hydroxysulphates linked with a sulphur β-D-glucopyranose) that can be aliphatic, indolic or aromatic depending on the side chain. When plant tissue is damaged, glucosinolates (GR) are hydrolysed by myrosinase (MYR) (thioglucoside glucohydrolase, EC 3.2.3.147), an enzyme present in *Brassica* vegetable as well. Among the breakdown products isothiocyanates are components known for the anticarcinogenic properties of *Brassicaceae* (Verhoeven *et al.*, 1997). In particular sulforaphane, isothiocyanate formed hydrolysis of an aliphatic glucosinolate, glucoraphanin (GR), shows the highest bioactivity (Mithen *et al.*, 2003).

Drying is used to extend the shelf life of vegetables and to reduce the mass for transportation. The low water activity prevents growth of microorganisms, enzymatic reactions, and other deteriorative reactions. Drying is an energy intensive operation for which the efficiency increases with raising temperatures. During drying of *Brassica* vegetables the heat load reduces the retention of the nutritional components glucosinolates and vitamin C (Vc) and the activity of myrosinase (MYR). Vega-Galvez *et al.* (2009) showed for convective drying with air that with temperatures in the range of 50°C to 90°C less than 40% of Vitamin C was retained and that the retention decreases with increasing temperature. Similar results were reported by Goula and Adamopoulos (2006) and Zanoni *et al.* (1998) for the degradation of Vitamin C during drying. Air drying reduced the indolic glucosinolates in broccoli by 15% and 46% when drying at 50˚C and 100˚C respectively (Mrkic *et al.,* 2010). Verkerk *et al.* 2004 showed MYR inactivation by thermal treatments, but no results are available on the effect of drying on MYR degradation.

Mild drying conditions are preferred to retain the nutritional components Vc and GR and the activating enzyme MYR. These mild conditions, however, conflict with the aim for a beneficial energy efficiency. In a previous study (Jin *et al.*, 2013) it was shown that with optimal trajectories of the control variables in time, the nutritional components Vc and GR are retained in combination with an increased energy efficiency. In this work the retention of Vc, GR and MYR during drying is further analysed by simulation and validation experiments. The results concern drying of fresh and blanched broccoli florets dried with different drying trajectories and for which the retention of Vc, GR and MYR are compared to drying under constant conditions.

Materials and methods

The materials and methods section considers a simulation and dynamic optimization part (*i.e.* degradation and drying kinetics, an optimization) as well as the description of the experimental procedures (*i.e.* sample preparation and determination of nutritional compounds) applied.

Degradation and inactivation kinetics

Drying trajectories are understood from temperature-moisture content state diagrams. The projection of the degradation rate constants of Vc, GR and MYR in the state diagram show that trajectories for optimal drying circumvent areas with high degradation rate constant values (Jin *et al.*, 2013). Only degradation rate constants of first order degradation can be projected in the state diagram. Oliviero *et al.* (2013)(**Chapter 4**) modelled the MYR inactivation as a consecutive reaction system. To project MYR inactivation in the state diagram a first order inactivation model is used in this work (see Appendix B).
The first order degradation/inactivation kinetics is given by:

$$\frac{dC}{dt} = -k_c C \qquad \textbf{Equation 5.1}$$

where C the fraction (-) of the remaining nutritional components and enzyme compared to the initial concentration, and k_c the degradation rate constant (1/s) that is temperature and moisture content dependent.

Expressions and constants for the degradation rate constant are given in Table 5.1. Vitamin C degradation depends on the properties of the food matrix and therefore different expressions for the degradation constant are found in the literature. The expression given by Mishkin *et al.* (1983) is most close to experimental results on broccoli and will be used in simulation and optimization in this work.

Table 5.1: Degradation kinetic models for Vitamin C and glucosinolates and inactivation kinetic model for myrosinase used in this work.

Compounds and Models reference	**Model**	**Parameter values**
Vitamin C (Mishkin *et al.*, 1983)	$k_c = \exp(a_1 X + \frac{a_2}{T^3} + a^3 X^3 + a_4 \frac{X^2}{T} + a_5 \frac{X}{T^2} + a_6 \frac{X^3}{T^3} + a_7$	a_1=17.94, a_2=-2.245 x 10^8 a_3=-33.33, a_4=5921 a_5=1.585 x 10^6 a_6=4.711 x 10^8, a_7=-2.239
Vitamin C (Mishkin and Saguy , 1984, Karim and Adebowale, 2009)	$k_c = k_{c,0} \exp\left(-\frac{E_{ca}}{RT}\right)$ $k_{c,o} = \exp\left(P_1 + P_2 X + P_3 X^2\right)$ $E_{ca} = P_4 + P_5 X + P_6 X^2 + P_7 X^3$	P_1=16.38, P_2=1.78 P_3=1.89, P_4=14831 P_5=241.1, P_6=656.2 P_7=236.8
Glucosinolates Oliviero *et al.,* 2012 - Chapter 3)	$k = k_{G,0} \exp\left(-\frac{E_{ca}}{RT}\right)$ $k_{ref} = \exp\left(u_1 + u_2 X\right)$ $E_a = u_3 + u_4 X + u_5 X^2$	u_1=25.21, u_2=8.29 u_3=91741, u_4=133.6 u_5=32606
Myrosinase (Oliviero *et al.*, 2013 - Chapter 5)	$k_M = k_{M,ref} \exp\left\{\left(\frac{E_{a,M}}{R}\right)\left(\frac{1}{T_{ref}} - \frac{1}{T}\right)\right\}$ $k_{M,ref} = y_1 X^3 + y_2 X^2 + y_3 X + y_4$ $E_{a,M} = y_5 X^3 + y_6 X^2 + y_7 X + y_8$	y_1=-1.88, y_2=2.32 y_3=-0.54, y_4=0.15 y_5=9.5 x 10^5, y_6=1.7 x 10^6 y_7=9.1 x 10^5, y_8=1.9 x 10^5

Oliviero *et al.* (2012, 2013) (**Chapter 3 and 4**) present the degradation of GR and inactivation of MYR. The degradation/inactivation rate constant is given as a function of temperature content at each moisture level. The data from Oliviero

et al. (2012, 2013) (**Chapter 3 and 4**) are processed into expressions in which the constants are given as a combined function of temperature and moisture (Table 5.1). The resulting degradation/inactivation rate constants for GR and VR and the degradation rate constant for Vc (Mishkin *et al.*, 1983) are projected in the isokinetic temperature-moisture content state diagram by contour lines of equal degradation rate constant (Figure 5.1). Degradation and inactivation is simulated with MATLAB.

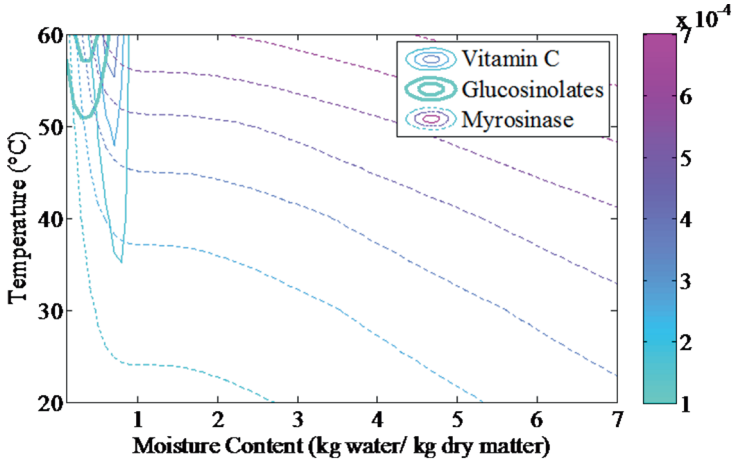


Figure 5.1: Temperature-moisture content state diagram of broccoli with contour lines for the degradation rate constants.

5

Drying kinetics

The mass balances for moisture in product and moisture in air, and the heat balance for air and product during drying are given by:

$$\frac{dX}{dt} = -k\left(X - X_e\right) \qquad \textbf{Equation 5.2}$$

$$0 = F_a\left(X_{a,in} - X_a\right) + M_p\frac{dX}{dt} \qquad \textbf{Equation 5.3}$$

$$0 = \left(C_{pa} + X_{a,in}C_{pv}\right)T_{in} - \left(C_{pa} + X_aC_{pv}\right)T - \Delta H_{vap}\left(X_a - X_{a,in}\right) \qquad \textbf{Equation 5.4}$$

X is the moisture content (kg water.kg dry matter^{-1}), X_e is the equilibrium moisture content (kg water.kg dry matter^{-1}), k is the drying rate constant (s^{-1}), F_a is the air mass flow rate (kg.s^{-1}.m^{-2}), X_a is the moisture content of air (kg water.kg dry air^{-1}), $X_{a,in}$ is the moisture in inlet air (kg water.kg dry air^{-1}), and M_p is the weight of dry matter

($kg.m^{-2}$), T is the product temperature (°C), T_{in} is the air inlet temperature (°C), C_{pp} is the specific heat capacity of product ($kJ.kg^{-1}.C^{-1}$), C_{pa} is the specific heat capacity of air ($kJ.kg^{-1}.C^{-1}$), C_{pv} is the specific heat capacity of water vapour ($kJ.kg^{-1}.C^{-1}$), $\Delta H_{v\alpha p}$ is the latent heat of vaporization ($kJ.kg^{-1}$).

The dryer and product properties used for calculations and optimization are the same as our previous work (Jin *et al.*, 2012) and are given in appendix A.

Objective function and constraints

Drying trajectories present the course of the drying conditions as a function of time. In our previous work drying trajectories are derived by optimizing piece wise constant or piecewise linear functions of the control variables in time (Jin *et al.*, 2013). Piecewise linear functions yield the best result for the retention for components and are applied in this work.

The moisture content at the end of the drying period is constrained by a requested final time value. Retention of Vc, GR and MYR close to 1 is aimed, which is reflected by the objective function:

$$\min J = \sum_{i=Vc,\mathrm{GR,MYR}} \left(w_i \left(\frac{C_i}{C_{i,0}} - 1 \right)^2 \right) \qquad \textbf{Equation 5.5}$$

w_i is a weight factor for each component in the objective function. All the elements in the objective function are relative values. Differences between the components are aimed to be small and therefore all weight factors are chosen to be 1. The control variable is the inlet air temperature which is constrained between 30 and 60°C.

The optimization was performed by using the MATLAB function *fmincon.*

Samples preparation

Fresh broccoli was purchased from a local market in Wageningen the same day of each drying treatment. Before each treatment, broccoli was chopped (pieces size approximately 2×2×2 cm).

Steam blanching

Broccoli florets were steam-blanched in a steam oven (DG 1050, Miele). First the oven was preheated to 100°C, then 100 grams of samples was placed in it for 1 min, afterwards, broccoli was cooled down in ice, and extra surface moisture was removed by paper tissue.

Drying treatments

A pilot batch dryer was used. Details of the dryer configuration are given in the work of Atuonwu *et al.* (2013). Samples were placed on a mesh tray (20cm×20cm), which was connected to a scale, to measure the water loss continuously. Every batch of drying experiments started with a sample mass of 100 grams. Temperature and airflow rate were controlled. Drying experiment where performed on fresh and steam-belched broccoli. Drying experiments continued until the moisture content of the samples reached equilibrium. Steam-blanching and drying experiments were performed in duplicate.

Glucoraphanin determination

The method described by Oliviero *et al.* 2012, (**Chapter 3**) based on extraction of glucosinolates with hot methanol as solvent was used with minor modifications. The sample size of the dried broccoli samples was approximately 0.1 g dry matter. The extracted glucosinolates were desulphated and then analysed according to Oerlemans *et al.* (2006). The desulfoglucosinolates were separated using a Lichrospher 100 Merck column (RP-18, 5µm) with a flow rate of 1 ml/min, and injection volume of 20 µl. Elution of desulfoglucosinolates from the HPLC column was performed by a gradient of water and acetonitril. GR was identified from the retention times and spectra of known GR present in reference *Brassica* samples.

Myrosinase determination

MYR was extracted according to Oliviero *et al.* 2013 (**Chapter 4**). To extract MYR from the broccoli matrix, the samples (equivalent to 0.05 g dry matter) were mixed in 140 mL of potassium phosphate buffer (50 mM pH 7.0) at a stirring plate at 15 ˚C overnight. MYR solutions were centrifuged at 3500 rpm for 10 min and the supernatants were filtrated (folded filters Grade 595 ½- 4-7 µm, Whatman) to further clean the solution. Then, MYR was purified and concentrated by using

filter tubes (Amicon Ultra-4 cut-off 30kD, Millipore) to remove components with molecular weight lower than MYR (sugars, glucosinolates etc.). The concentrated enzyme was dissolved again in 0.5 ml potassium phosphate buffer (50 mM pH 7.0). The MYR activity was determined according to a coupled enzymatic procedure as described by Van Eylen *et al.* (2006), with some modification. In this assay the D-glucose formed by the reaction between MYR and sinigrin as a substrate could be used to transform NADP+ to NADPH (D-Glucose kit, Enzyplus, Biocontrol). The reaction mixture consisted of 0.9 ml of a water solution containing 0.05 g/l MgCl2 and 1 g/l ascorbic acid, 50 µl of the extracted MYR solution, 50 µl of test kit solution R1 (imidazole buffer, magnesium chloride and sodium azide), 50 µl of test solution R2 (NADP+, ATP), 5 µl of test solution R3 (hexokinase (300 U/ml), glucose-6-phosphate dehydrogenase (400 U/ml) and 50 µl of sinigrin solution (30 mg/ml). The formation of NADPH was followed by a spectrophotometer (Cary UV 50, Bergen op Zoom, The Netherlands) at 340 nm for 7 min. The activity was determined based on the slope of the linear part of the curve of absorbance versus reaction time.

Total vitamin C determination

The total Vc concentration, (ascorbic acid added up to dehydroascorbic acid), was analysed according to Hernandez *et al.* (2006) and Wechtersbach and Cigic (2007) with modification. Samples (equivalent to 0.05 g dry matter) were mixed with 3.5 ml of metaphosphoric acid (MPA), tert-butylhydroquinone (THBQ) solution, (3% MPA, 1 mM THBQ in Milli-Q water), by using an Ultra Turrax. After 5 min at 3000 rpm at 4˚C, the supernatant was collected in a new tube. The pellet was re-extracted with 3.5 ml of the MPA, THBQ solution, twice, and after centrifugation the supernatants of each sample were collected in the same tube. To determine the total Vc, the 15 µL of Tris-2-carboxyethyl phosphine solution (1M in Milli-Q water) was added to 1.485 ml of the prepared extract as described above. After incubation in dark at room temperature for 20 minutes the samples were analysed by HPLC (Varian Polaris column, C18, 4.6 x 150 mm). With a flow rate of 1 ml/min, an injection volume as 20 µl. Elution of was performed by a gradient of Orthophosphoric Acid 0.2% in milli Q water.

Results and discussion

First, simulation results for a constant temperature are compared with optimized temperature trajectories. Then, for validation two sets of drying experiments were performed for broccoli: 1) at constant temperature, and 2) with optimized temperature trajectories.

Simulation at constant temperature

Simulation results for the degradation of Vc, GR and MYR during drying at 40°C and 50°C are given in Figure 5.2. The time window for the simulations concerns the time needed to realize a final moisture content of 0.1 kg water.kg dry matter^{-1} and depends on the applied drying strategy. GR is the most stable compound compared to MYR and Vc. Retention of GR is over 70% after 24 hours drying at 50°C.

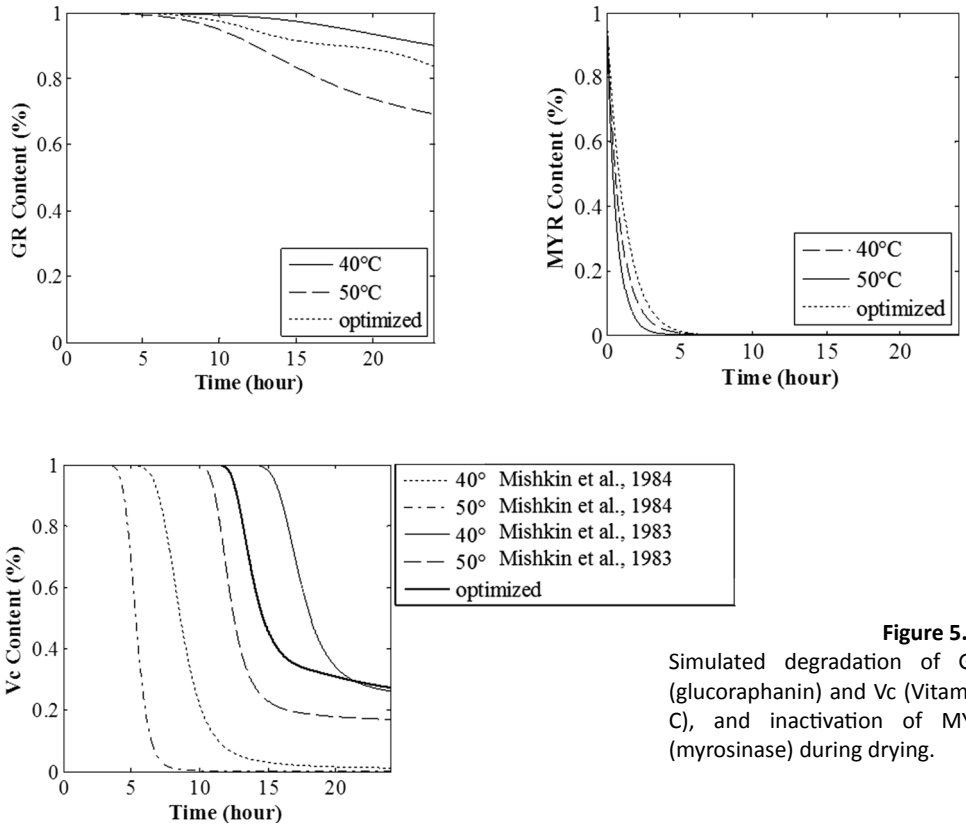


Figure 5.2: Simulated degradation of GR (glucoraphanin) and Vc (Vitamin C), and inactivation of MYR (myrosinase) during drying.

For Vc the two models from Table 5.1 are simulated. The Vc degradation for the model given by Mishkin *et al.* (1984) is faster than the earlier presented model by Mishkin *et al.* (1983). Both models have in common that Vc degradation at high moisture content is slow. Due to the high degradation rate constants in the moisture content range between 0.5 and 1 kg water.kg dry matter^{-1} (Figure 5.1), Vc degrades for both models in a short time when the product passes this region.

MYR is most heat sensitive compared to GR and Vc. With the high inactivation rate constant the inactivation of MYR occurred in a short time. After 3-4 hours there is hardly any activity left.

The simulated product temperature trajectory for the inlet air temperature of 50°C is given in the state diagram (Figure 5.3). The diagram shows how the product degrades by passing the heat sensitive areas.

Optimized drying trajectories

The optimal product temperature trajectory is given in Figure 5.3. The simulated degradation of Vc, GR and MYR is also presented in Figure 5.2. Moreover, the product trajectory is plotted in the temperature-moisture content state diagram (Figure 5.3).

Although Vc and GR are stable in the high moisture content range, MYR is very sensitive to intensive heating. Therefore, the inlet temperature trajectory started with a relatively low temperature and increases gradually with decreasing moisture content (for the first and second stage; *i.e.* the first two piecewise linear functions). At the end of the second stage the product approaches the moisture-temperature area with raised degradation rates for Vc. Here the temperature trajectory responds by decreasing the temperature. To satisfy the constraint on the final moisture content the temperature increases to a higher value in the final stage, where the degradation rates for all the components are low.

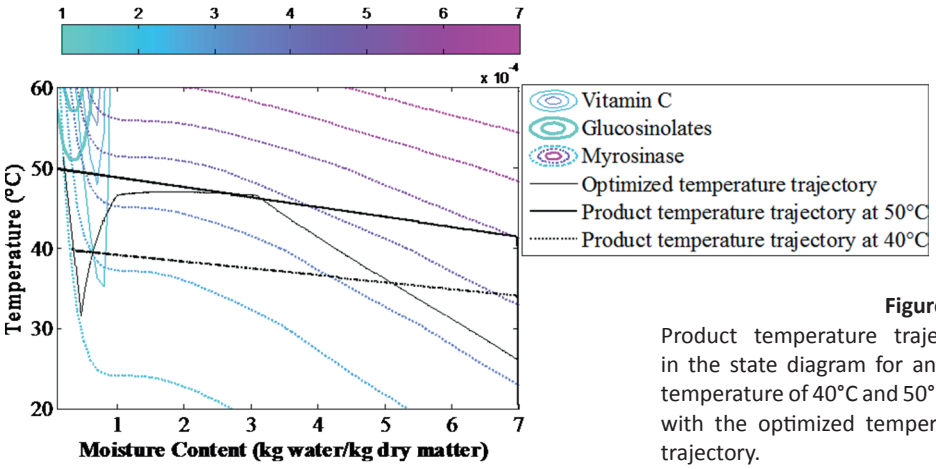


Figure 5.3: Product temperature trajectory in the state diagram for an inlet temperature of 40°C and 50°C and with the optimized temperature trajectory.

Due to the constraints on final moisture content and drying time, the optimized drying trajectory cannot fully avoid passing through the region of the high degradation rates. Although the residence time in the high degradation rate region has been minimized, it still results to some degradation for all the components, especially for Vc and MYR.

Comparison of the product trajectory for drying at constant temperature of 50°C and the time varying trajectory show clearly the difference between the methods and the advantage of a time varying strategy.

From the degradation kinetics, it can be expected that influence of drying on GR degradation is minimal whereas MYR is very sensitive to heat treatment. According to the simulation results based on optimized temperature trajectory, the estimated retentions are 83%, 0, 28% for GR, MYR and Vc, respectively.

Compared to drying at 50°C the application of the optimal drying trajectory increased the estimated retention of Vc with 60% (from 18 to 28% of the initial concentration). This result is a bit below the simulated outcome of the previous optimization work where a retention of Vc content by 55% was predicted (Jin *et al.*, 2012). In that case, peeled broccoli stalks were dried and the peeled stalks, due to the removal of the mass transfer barrier, can be dried much faster compared to fresh broccoli floret. Since the total loss of nutritional components is dependent on both degradation rate and time at a moment during drying. The extended drying time may lead to significant degradation of these components.

Degradation and inactivation at constant drying conditions: experimental results

The loss of glucosinolates during water blanching is often the result of leaching rather than temperature degradation (Vallejo *et al.* 2002; Oliviero *et al.,* 2013). Steam blanching was applied as drying pre-treatment to prevent GR, Vc and MYR loss to the water. Just as in the work of Vallejo *et al.* (2002) and the work of Verkerk *et al.* (2010) short steam blanching did not reduce GR content in this work. In fact, GR retention was 104%. The average values, for GR, MYR and Vc, were calculated among all the steam blanching treatments that were performed on different batch of broccoli. Thus the steam blanching treatment was performed before the drying at 40˚C and before drying at 50˚C. The drying treatments at each temperature, were performed in duplicates. The slight increase of GR may be explained by the break-down of plant tissue caused by heat treatments, which results in a better release of these components during the extraction (Verkerk *et al.,* 2010). Drying fresh and steam blanched broccoli at 40˚C and 50˚C resulted in 100% GR retention. For dried broccoli previously steamed, the retention of GR was calculated taking as initial GR, the GR after steaming. Mrkic *et al.* (2010) found a comparable high retention of indolic glucosinolates (83-90 %) in broccoli dried at 50˚C. The GR retention upon drying at optimal temperatures trajectory was as high as upon drying at constant temperatures.

According to the experimental results, MYR is more heat sensitive than GR and Vc (Table 5.2). The steam blanching pretreatment strongly affected the final MYR activity. After one minute steam blanching, the MYR activity retention was by 30%. Verkerk *et al.* (2010) found nearly 90% of MYR activity after broccoli steaming. In that study broccoli samples were larger than in the present study and a higher amount of broccoli was processed in the steamer that could have led to a reduced heat load average. MYR activity in fresh and steam blanched broccoli was retained by 31% upon drying at 40˚C and by 15%. upon drying at 50˚C. Although a high deviation was found for both drying temperatures, the trend for fresh and steam blanched broccoli is clear: the higher the drying temperature, the lower the MYR activity. The large deviation of MYR activity for both the steam blanched and dried samples can be explained by the large deviation of physical properties of broccoli. In addition, MYR activity in one broccoli head may vary among the florets, which increases the variation in MYR activity. Finally, Table 5.2 shows that applying the

optimal temperature trajectory resulted in the highest MYR-retention with a good reproducibility over the experiments.

Upon steaming, Vc was retained by 126%. It is known that Vc is a heat sensitive compound that can be degraded upon cooking (Pellegrini *et al.*, 2010), however, similar to GR, the increased Vc content could be explained by the breaking down of plant tissue caused by heat treatments that can result in a better Vc extractability. Other mild cooking treatments, as 1 min steam blanching, are also known to increase the antioxidant compound in vegetables (Turkmen *et al.,* 2005). The retention of Vc after drying at 40˚C is comparable with the ones dried at 50°C (Table 5.2). The application of the optimal temperature trajectory yield also for Vc the highest retention. Optimization seems thus attractive to retain the heat sensitive components.

The simulation results by the optimized temperature trajectory underestimate the experimental retention (by the optimized temperature trajectory) of GR, MYR and Vc (Table 5.2). According to the simulation of the optimized temperature trajectory, the retention was 83%, 0 % and 28% for GR, MYR and Vc, respectively. However, the experimental retention was 101% for GR, 55% for MYR and 85% for Vc. Also the simulation at constant conditions underestimated GR and MYR retention (Table 5.2).

The current drying experiments concerned intact broccoli florets with glucosinolates and Vc localised in the vacuoles and MYR localized in special vacuole-like myrosin cells. On the contrary, the simulation results are based on kinetics, for degradation of GR and inactivation of MYR, measured on powdered freeze dried samples with different moisture content that have been exposed to a temperature treatment in sealed heating tubes (Oliviero *et al.,* 2012, 2013) (**Chapter 3 and 4**). The release of the compounds may have enhanced their degradation or inactivation, whereas the plant structure may have had a protective effect on GR and MYR during drying experiments. The different heat treatments may also have affected GR degradation and MYR inactivation. In fact, drying can be considered as a dynamic process in which the water evaporation leads to an increase in concentrations of reactants and development of a concentration gradient. In addition, in intact broccoli also transport due to the capillary action may occur. These changes in concentration will influence the reaction kinetics. Regarding Vc, the kinetics of degradation was

studied in a model system that consisted of inert compounds added of a known Vc concentration, and the heating treatments were performed in closed tubes placed in controlled temperature water bath. Besides the consideration mentioned on using different heating treatments (drying *vs*. heating in closed tubes), the type of model system may further affect the mechanism of a reaction. In fact, the specific composition of a food matrix, as real system, has a large impact of the kinetics of a reaction as compared to a relatively simple model system (Van Boekel, 2009). The possible effects of the food matrix on reaction kinetics are various, *e.g.*, molecular crowding or volume exclusion, buffering effect of some compounds and presence of ionic or nonionic solutes (Van Boekel, 2009).

Table 5.2: Comparison of mathematical simulation results and experimental results of drying. Glucoraphanin (GR), myrosinase (MYR), vitamin C (Vc). The values of the experimental results, are the average of two drying experiments (duplicate) ± standard deviation.

Retention (%)	**Simulation results**			**Experimental results**		
	Drying at Constant Temperature		Drying at Optimized Trajectory	Drying at Constant Temperature		Drying at Optimized Trajectory
	40 ˚C	50 ˚C		40 ˚C	50 ˚C	
GR	85	70	83	100±2.30 98±1.1*	108±4.10 96±4.2*	101±1.10
MYR	0	0	0	29±5.3 33±1.0*	19±13 12±13*	55±3.1
Vc	0# - 28•	0# - 18•	28	60±44 52±32*	66±17 54±17*	85±13

*broccoli previously steamed
model of Mishkin *et al.*, 1984
• model of Mishkin *et al.*, 1983

In line with the findings of other investigations (Lewicki, 2009, Jin *et al.*, 2012) changes in the physical state of the product are important during drying, and these changes might have a role in how the components in the plant structure react on each other, therefore, modelling of degradation of these nutritional components should be coupled with other physical models that take into account the internal structure changes during processing such as physics based shrinkage. Anyhow, these results show that the use of optimized temperature trajectory and state diagram was an efficient tool to design air drying process to produce broccoli with the desired characteristics.

Conclusions

In the first part of this work, the impact of the degradation kinetics during drying of GR, MYR and Vc was analysed theoretically and experimentally at two temperatures (40°C and 50°C) for fresh and steamed broccoli florets. Degradation rate was found to be much smaller than predicted from kinetic data, especially for MYR. In the second part of this work, optimal drying trajectory for the temperature profile was developed to minimize the degradation of these nutritional components. Representation of the trajectory in temperature moisture content state diagram showed that the strategies avoid temperature moisture content regions where degradation rate is high or to avoid long residence time in that region. The estimated retention according to the optimized temperature trajectory was 83%, 0% and 28% for GR, MYR and Vc, whereas retention showed by the experimental results 100% for GR, 55 % for MYR and 84% for Vc (average values).

The discrepancy between the experimental and simulation results, for both drying at constant temperature and drying with optimized temperature trajectory, was explained by the different physical states of the samples (intact sample and powder) and by the different heating treatments applied to measure the reaction kinetics for the simulations results. This finding also suggests that besides temperature and moisture content, changes in the physical state that occur during drying also play an important role in the degradation of nutritional components during drying.

Furthermore, the experimental results confirmed that with the optimized drying trajectory, retention of these healthy components can be significantly improved compared to the drying at constant conditions (50°C). Despite a mismatch in the kinetic expressions and the degradation behaviour in the broccoli matrix this work shows that using a mechanistic assisted optimization is a good approach for process design to improve the quality of high value food products. Hereby the state diagram with degradation rates supports the understanding of the drying strategy.

Acknowledgement

This work is supported by the Energy Research Program EOS (EOS LT07043) of the Dutch Ministry of Economics.

Appendix A Modelling and Optimization

The modelling and optimization of the drying process concern uniform pieces of broccoli floret that pass a dryer system. The product and drier properties are specified in Table A1.

Table A1: Dryer and product properties used for calculations and optimization

Parameter	Value
M_p	1.00 (kg.m^{-2})
$T(t=0)$	25°C
C_{pa}	1.00 (kJ.kg^{-1}.K^{-1})
C_{pv}	1.93 (kJ.kg^{-1}.K^{-1})
C_{pp}	0.837+1.256X(kJ.kg^{-1}.K^{-1}) (Hussain & Dincer, 2003)
ΔH_{vap}	2500-2.386T (kJ.kg^{-1}) (Henderson-Sellers, 1984)
T_{amb}	20 (°C)
k	$5.94\exp(-\frac{31265}{8.314T})(s^{-1})$
$X_{a,in}$	0.007 (kg water/kg dry matter)

The objective of the optimization of the drying process is to minimize the loss of the nutritional components vitamin C (Vc), myrosinase (MYR) and glucosinolates (GR). The control variables considered were temperature of the inlet air. The control variables are constrained by:

$$30\,^{\circ}\mathrm{C} \leq \mathrm{T_{in}} \leq 60\,^{\circ}\mathrm{C}$$

And air flow rate is set to be constant:

$$Fa = 0.1\frac{kg}{s}$$

The restriction on the control variable air temperature affects the degradation rate

constants. Drying trajectories can be calculated as continuous functions (Bryson, 1999) or by discrete functions like piecewise constant and piecewise linear functions. Discrete functions are most suitable for realization in batch or continuous drying of vegetables; each function can represent a drying stage. The time intervals/drying stages were chosen to be equally. In our previous work it has shown that piecewise linear functions resulted in the best performance with 55% for vitamin C retention and 65% for energy efficiency. These results are superior to a dryer with the best constant settings of the controlled variables: 32% retention for vitamin C and 28% of energy efficiency. In other ways, the energy consumption can be halved and the retention of nutritional components increased with 70%. These results show the impact of using optimized drying strategies instead of constant drying conditions. Therefore in this work piecewise linear with four stages controlled trajectory for inlet air temperature was chosen for the optimization problem.

Appendix B degradation kinetics of myrosinase and glucosinolates

The degradation kinetics for glucosinolates and myrosinase used in this work were derived from works of Oliviero *et al.* (2012) (Chapter 3) for glucosinolates, and Oliviero *et al.* (2013) (Chapter 4) for myrosinase respectively. According to Oliviero *et al.* (2012), for glucosinolates first order degradation kinetics is used.

$$\frac{\mathrm{d}C}{\mathrm{d}t} = -k_c C$$

$$k_i = k_{i,ref} \exp\left\{\left(\frac{E_{a,i}}{R}\right)\left(\frac{1}{T_{ref}} - \frac{1}{T}\right)\right\}$$

$$T_{ref} = 373K$$

Since glucoraphanin is the dominant glucosinolate the degradation kinetics for glucoraphanin is considered. The activation energy and reference rate constant according to their work is listed in Table B1.

Table B1: Activation energy, and rate constants at reference temperature for glucoraphanin (first order reaction kinetics).

Water Content content (%)	13	34	56	68	82
$E_a \pm \mathrm{SD}\left(\frac{\mathrm{kJ}}{\mathrm{mol}}\right)$	159±7	96±5	99±3	98±3	113±3
$k_{d,100^\circ\mathrm{C}} \pm \mathrm{SD}$ $\left(\times 10^{-2}\mathrm{min}^{-1}\right)$	2.7±0.3	6.7±0.4	4.1±0.3	0.9±0.2	1.5±0.1

To use the degradation kinetics model in combination with the drying model, the reference rate constant ($k_{i,ref}$) is written as a function of temperature and moisture content (Table 5.1 and Jin *et al.*, 2012).

According to Oliviero *et al.* (2013) (Chapter 4), MYR inactivation follows a consecutive step reaction order. In the first step, the native enzyme (N) structure changes to an intermediate form (Y) with lower activity, following by a second reaction to fully inactive the enzyme, both reactions are irreversible.

$$N \xrightarrow{k_1} Y \xrightarrow{k_2} I$$

$$\frac{\mathrm{d}C_N}{\mathrm{d}t} = -k_1 C_N, \quad \frac{dC_Y}{dt} = -k_2 C_Y + k_1 C_N$$

$$\frac{dC_I}{dt} = k_2 C_X$$

$$k_i = k_{i,ref} \exp\left\{\left(\frac{E_{a,i}}{R}\right)\left(\frac{1}{T_{ref}} - \frac{1}{T}\right)\right\}$$

$$T_{ref} = 323K$$

The results of Oliviero *et al.* 2013 (Chapter 4) for four levels of moisture content at different temperatures are listed in Table B2.

Table B2: Activation energy, and degradation rate constant at reference temperature for myrosinase (consecutive step reaction order).

Water Content (%)	$k_{1,50\,^{\circ}C}$ ± SD (x 10^{-2} min^{-1})	$k_{2,50\,^{\circ}C}$ ± SD (x 10^{-2} min^{-1})	E_{a1} ± SD (kJ/mol)	E_{a2} ± SD (kJ/mol)	Y± SD (%)
10	0.06 ± 0.02	0.0	140 ± 18	NE	16 ± NE
31	5.1 ± 1.1	0.9 ± 0.3	59 ± 20	16 ± 29	43 ± 19
67	4.8 ± 4.0	1.2 ± 0.4	51 ± 13	62 ± 28	37 ± 2
90	5.5 ± 1.2	5.7 ± 8.5	44 ± 16	120 ± 124	25 ± NE

To present the inactivation kinetics in a state diagram, the results of Oliviero *et al.* 2013 (Chapter 4) are approximated by a first order inactivation model.

$$\frac{dC_M}{dt} = kC_M$$

The degradation rate constant will be:

$$k_M = k_{M,ref} \exp\left\{\left(\frac{E_{a,M}}{R}\right)\left(\frac{1}{T_{ref}} - \frac{1}{T}\right)\right\}$$

The reference temperature used in this work is 373K.

In vivo formation and bioavailability of isothiocyantes from glucosinolates in broccoli as affected by processing conditions

Oliviero T.
Verkerk R.
Vermeulen M.
Dekker M.

Submitted for publication

CHAPTER

Abstract

The aim of this study was to investigate the effect of remaining myrosinase (MYR) activity in differently processed broccoli on sulforaphane (SR) and iberin (IB) isothiocyanate formation, bioavailability and excretion in human volunteers were studied. For this purpose, Five different broccoli products were obtained with similar glucoraphanin (GR) and glucoiberin (GI) content, yet different MYR activity. Excretion profiles of conjugates of SR and IB in urine was measured in 15 participants after ingestion of the broccoli products. A reduction of 80% of MYR in the product did not cause any differences in the total amount of SR and IB found in urine compared to the product with still 100% MYR. Complete inactivation of MYR gave the lowest total amount of SR and IB in urine (10% and 19%). A remaining MYR of only 2% in the product gave an intermediate amount (17% and 29%). The excretion half-lives of SR and IB conjugates were comparable for all the products (2.5h on average), although the maximum excretion peak times were clearly shorter when the remaining MYR was higher (2.3-6.1h). For the first time the effect of remaining MYR activity on ITC bioavailability was systematically and quantitatively studied. Processing conditions have a large effect on the kinetics and total bioavailability of ITC's from broccoli. These results can be used to improve products and processes from a health perspective.

Introduction

The protective effect of vegetables against many types of cancer, is well known (Steinmetz & Potter, 1996). The seasonal nature of vegetable production has called for the use of food processing, such as drying, freezing, allowing consumers to find these vegetables on the market anytime. As a result, people often consume processed vegetables. This is the case of many type of *Brassica* vegetables. Among those, broccoli (*Brassica oleracea* var*. italica*) is often consumed cooked. But cooking or other processes can also reduce the occurrence of those compounds that are accountable for the healthiness of such vegetables.

The health-promoting effect of *Brassica* vegetables has been ascribed in part to glucosinolates (GLs) occurrence (Wallenberg, 1992). GLs are a group of secondary plant metabolites (β-thioglycoside N-hydroxysulphates linked with a sulphur β-D-glucopyranose) and depending on the side chain GL can be classified as aliphatic, aromatic or indolic. GL can be hydrolyzed by myrosinase (MYR) (thioglucoside glucohydrolase, EC 3.2.3.147), an enzyme present in *Brassica* vegetables as well. The breakdown products are various biologically active metabolites, including isothiocyanates, thiocyanates and nitriles. Among the breakdown products isothiocyanates (ITC) are accountable for the anticarcinogenic properties of *Brassicaceae* (Verhoeven *et al.*, 1997).

Food processing can reduce GL concentrations, MYR activity and in turn, ITCs formation and their intake by humans (Dekker, *et al.*, 2000). During heat treatments several mechanisms take place, in particular high temperatures cause GL degradation and MYR inactivation (Rungapamestry *et al.*, 2006). GLs are stable during mild cooking, but their content may be reduced by leaching into the cooking water (Pellegrini *et al.*, 2009; Verkerk & Dekker, 2004). MYR is often inactivated even during mild cooking (Verkerk *et al.*, 2010). As a consequence, broccoli is mainly consumed without MYR activity.

In cooked vegetables where GLs are still present and MYR is inactivated, GLs hydrolysis occurs during digestion. In fact during digestion, the GLs hydrolysis is mediated by MYR-like activity of the enteric microflora, but to a lesser extent than in plant (Shapiro, 1998).

To enhance the health effect of broccoli consumption, scientists have bred broccoli with high GLs content. For instance the superbroccoli, Beneforte®, with higher glucoraphanin concentration. Glucoraphanin is an aliphatic GL that is the precursor of sulforaphane, the ITC that shows the highest potency to induce phase II detoxification enzymes in humans (Mithen *et al.*, 2003).

ITCs uptake, both as fraction formed by MYR present in the vegetable and as fraction formed by MYR-like activity of the enteric microflora, can be assessed by measuring the ITC conjugates excreted in urine. In the human body absorbed ITCs are conjugated to glutathione, further metabolized to ITC conjugates, and subsequently excreted in urine (Shapiro *et al.* 1998, Mennicke *et al*. 1988, Vermeulen *et al*. 2008). Many investigations on this topic focus on ITC conjugates excreted in urine after consumption of different forms of broccoli (1) raw broccoli where MYR is still active, (2) cooked broccoli where MYR is inactivated (Shapiro *et al.*, 1998; Shapiro *et al.*, 2001; Rouzaud *et al.*, 2004) and (3) crushed broccoli where ITCs are already formed prior to consumption (Vermeulen *et al.*, 2006; Vermeulen *et al.*, 2008). No study investigated the influence of different MYR activity on ITC formation and uptake yet.

In the present study, a human intervention study was performed to investigate the formation of ITCs and their bioavailability in human volunteers, after consumption of differently processed broccoli products. A batch of Beneforte® broccoli, broccoli bred to have higher glucoraphanin concentration, was divided into five sub-batches and each sub-batch was processed in order to obtain batches with the same GLs content but different MYR activity. Then, 15 human volunteers consumed these broccoli products in a cross-over design study. Glucoraphanin (GR), glucoiberin (GI) and their ITCs, sulforaphane (SR) and iberin (IB) were determined in the broccoli products and their excreted metabolites were measured in urine. The role of different MYR activity in the broccoli products on ITC formation, bioavailability and excretion kinetics was investigated.

Materials and Methods

Participants

Fifteen apparently healthy adults (aged 26-50 years, body mass index 21 ± 2 kg/m^2, 6 men and 9 women, 13 Caucasian, 2 Asian and 1 Latin American), were recruited at Wageningen University. Each subject gave written informed consent after being informed about the study. The study was positively advised by an external Medical Ethical Committee and was conducted at the Food Quality and Design group at Wageningen University.

Study Design

Each participant joined 5 sessions at 10.00 a.m. with at least one day of washing out in between, in a randomized cross over trial. Every session the participant consumed one of the 5 different broccoli products along with 30 g raisin bun and ad libitum water. Participants were not fasted before the consumption of broccoli products. Participants were asked to not consume any other *Brassica* vegetables during the three weeks of treatments. Participants did a maximum of 2 sessions per week.

Broccoli product description and preparation

A batch of Beneforte® broccoli grown in open fields in The Netherlands, was harvested in December, 2012. The batch was divided into five sub-batches, then the edible florets (floret plus approximately 1 cm stalk) were separated from the stem, the florets were chopped into smaller pieces (size approximately 2×2×2 cm) and processed further.

Sub-batch 1: High MYR broccoli powder (High MYR BP) product.
Fresh florets were frozen and then freeze dried (GRInstruments, Model GRI 20-85 MP 1996, The Netherlands). The dried florets were ground to a fine powder by using a Waring blender (model 34BL99, Dynamics Corp. of America, New Hartford, CT, USA).

Sub-batch 2: High MYR broccoli florets (High MYR BF) product.
Fresh florets were frozen and then freeze dried.

Sub-batch 3: Medium MYR broccoli florets (Medium MYR BF) product.
Fresh florets (300 g) were heated in a microwave oven at 180 W for 8 min (DAEWOO, Model KOC-87-T Korea) to partially inactivate MYR. After cooling down on ice, broccoli was stored overnight at -20 ˚C. Then frozen broccoli was subsequently freeze dried.

Sub-batch 4: Low MYR broccoli florets (Low MYR BF) product.
Fresh florets (300 g) were heated in a microwave oven at 540 W for 3 min to partially inactivate MYR. After cooling down on ice, broccoli was stored overnight at -20 ˚C. Then frozen broccoli was subsequently freeze dried.

Sub-batch 5: No MYR broccoli florets (No MYR BF) product.
Fresh florets (300 g) were heated in a microwave oven at 900 W for 5 min to totally inactivate MYR. After cooling down on ice, broccoli was stored overnight at -20 ˚C. Then frozen broccoli was subsequently freeze dried. After freeze drying, the samples were stored at 4˚ C in sealed containers. All the broccoli products consisted of intact florets except the fresh broccoli powder (Sub-batch 1) product which was ground. A portion of 5 g of each broccoli product was served after 2 minutes rehydration with 90 ml of water at 40 ˚C, and with 30 g of raisin bun and water.

Urine collection

Volunteers were asked to drink sufficient water to collect urine frequently during the day. Urine samples (one spot urine) were collected before consumption of each experimental rehydrated broccoli product and during 22-23 h after consumption. Urine samples were collected in separate flasks and were kept refrigerated before analyses that were performed within 24 h.

GLs determination in the products

GLs determination was performed on fresh broccoli, after microwaving, after freeze drying and on the rehydrated broccoli products (broccoli and rehydration water). The method described by Oliviero *et al.* 2012, was used with minor modifications. Samples were ground with in liquid nitrogen by using a Waring blender (model 34BL99, Dynamics Corp. of America, New Hartford, CT, USA). After grinding, broccoli samples (0.1 g dry matter), were extracted with 3 ml hot (75 ˚C) methanol (100%) and 200 µL of 3 mM glucotropaeolin solution (internal standard) was

added. Samples were incubated in a water bath of 75°C for 25 min, and vortexed every 5 min. After incubation, the samples were centrifuged for 10 min at 2500 rpm. The supernatant was collected in 15 ml tubes. The pellet was re-extracted twice with 2.3 mL of hot methanol (70%), centrifuged and the supernatants were combined with the first supernatant. The GLs extracts and the rehydration waters from the rehydrated broccoli products were desulphated and then analysed by HPLC according to Oerlemans *et al.* (2006). In short, the desulfoglucosinolates were separated using a Lichrospher 100 Merck column (RP-18, 5µm) with a flow rate of 1 ml/min, an injection volume as 20 µl. Elution of desulfoglucosinolates from the HPLC column was performed by a gradient of water and acetonitrile.

MYR determination in the products

MYR was extracted according to Oliviero *et al.* 2013, (**Chapter 4**). MYR activity was determined in fresh broccoli, after microwaving, after freeze drying and on the rehydrated broccoli products (broccoli and rehydration water). Samples were ground in liquid nitrogen by using a Waring blender (model 34BL99, Dynamics Corp. of America, New Hartford, CT, USA). To extract MYR from broccoli matrix, samples (0.05 g dry matter) were added to 140 mL of potassium phosphate buffer (50 mM pH 7.0) and mixed by stirring plate at 15 ˚C overnight. The next day, the broccoli extracts and the rehydration water from the rehydrated broccoli products were centrifuged at 3500 rpm for 10 min and the supernatants were filtrated (folded filters Grade 595 ½- 4-7 µm, Whatman) to further clean the solution. Then, MYR was purified and concentrated by using filter centrifugation tubes (Amicon Ultra-4 cut-off 30kD, Millipore, centrifugation at 4000 g for 10 min) to remove the compounds with low molecular weight (sugars, GLs etc.). The concentrated enzyme was dissolved again in 0.5 ml potassium phosphate buffer (50 mM pH 7.0). The MYR activity was determined according to a coupled enzymatic procedure as described by Van Eylen *et al.* (2006), with some modification. In this assay the D-glucose formed due to the reaction between MYR and sinigrin as a substrate could be used to transform NADP+ to NADPH (D-Glucose kit, Enzyplus, Biocontrol). The reaction mixture consisted of 0.9 ml of a water solution containing 0.05 g/l $MgCl_2$ and 1 g/l ascorbic acid, 50 µl of the extracted MYR solution, 50 µl of test kit solution R1 (imidazole buffer, magnesium chloride and sodium azide), 50 µl of test solution R2 (NADP+, ATP), 5 µl of test solution R3 (hexokinase-300 U/ml), glucose-6-phosphate

dehydrogenase (400 U/ml) and 50 µl of sinigrin solution (30 mg/ml). The formation of NADPH was followed by a spectrophotometer (Cary UV 50, Bergen op Zoom, The Netherlands) at 340 nm for 7 min. The activity was determined based on the slope of the linear part of the curve of absorbance versus reaction time.

ITCs determination in the rehydrated broccoli products - SR and IB

The ITCs were measured after conjugation with 1-butanethiol as described before (Vermeulen *et al.*, 2008). To quantify the ITCs formed in the broccoli products before consumption, each product was prepared by rehydrating 5 g of dried broccoli with 90 mL of water at 40 ˚C. After two min, water and broccoli were separated. The water was kept on ice and the broccoli was ground while kept frozen by liquid nitrogen to prevent MYR to hydrolyze GLs during the ITC extraction. The ITC extraction and conjugation reaction was performed in methanol buffer to inactivate the MYR to prevent any further ITCs formation during the assay. The ground frozen broccoli (1 g) was extracted with 16 mL of buffer (formic acid 0.08 M, triethylamine 15.8 M in methanol) and after centrifugation the supernatant (2) was analysed. Water from broccoli rehydration and from broccoli extraction (200 µL) were mixed to 20 µL phenyl ITC (20mM) as internal standard and 800 µL of buffer (formic acid 0.08 M, triethylamine 15.8 M in methanol). Then, 12 µL of 1-butanethiol (99%), was added to the mixture and after incubation at 50 ˚C for 2 h, the samples were diluted with 4 mL of methanol, ready to be analysed. The n-butanethiol conjugates of sulforaphane and ibern were analysed by LC-MS/MS (TSQ Quantum, Thermo) with XBridge RP18 (3.0 x 100 mm, 5 µm) with pre-column. SR (LKT laboratories/ Biomol S8040 177.29 g/mole) and IB (LKT laboratories/Biomol I0416, Iberin 163,26 g/mole) conjugates were prepared as described for the samples and were used for quantification by external calibration.

SR and IB conjugates in urine determination

The ITCs conjugated were measured as described before (Vermeulen *et al.*, 2008). Urine (200 µL) was mixed to 20 µL phenyl ITC (10 µM) as internal standard 800 µL of buffer (formic acid 0.08 M, triethylamine 15.8 M in methanol) and 12 µL of 1-butanethiol (99%). After 2 h incubation at 50 ˚C the samples were diluted with 4 mL methanol, ready to be analysed. The n-butanethiol conjugates of sulforaphane and ibern were analysed by LC-MS/MS as described for ITC determination.

Statistical and Pharmacokinetic Analysis

Urinary excretion curves of IB and SR conjugates (excretion rate of IB and SR conjugates versus time) were fitted to a one-compartmental model (as initial value the max excretion peak was considered) with the assumption of first-order excretion kinetics. The excretion rate constants of each participant for each broccoli product, were estimated by the software package Athena Visual Workbench (www.athenavisual.com) and then expressed as excretion half-life $t_{1/2}$ (0.693/k). The maximum excretion peak time (t_{max}, h) was graphically determined from the urinary IB and SR conjugates excretion rates plotted versus time. Studies in literature report that an increase plasma concentration of ITCs conjugates results in an increase of ITCs conjugates urine excretion (Conaway *et al.*, 2009; Vermeulen *et al.*, 2006; Vermeulen *et al*., 2008) . The bioavailability was calculated as cumulative amount (µmol) of SR and IB conjugates excreted in 24 h urine divided by the GR or the GI amounts (µmol), respectively, in the consumed products (Vermeulen *et al.*, 2006; Vermeulen *et al.*, 2008). This calculation includes both the formation step as well as the actual bioavailability and excretion step. Statistical significance of the differences among GL and MYR concentration in the five broccoli products and the cumulative amount of SR and IB conjugates, bioavailability, $t_{1/2}$, t_{max}, was calculated with one-way ANOVA followed by Bonferroni's multiple comparison.

Results

GR, GI content and MYR in dry broccoli products

In order to obtain 5 broccoli products with different MYR activity but similar GR and GI contents, different processing conditions were applied (see Materials and Methods). GR and GI content in fresh broccoli was 2.2 ±0.8 µmol/g DW and 14 ±1 µmol/g DW. After processing, GR and GI concentration in all the 5 broccoli products did not change ($p > 0.05$). Hence, the intake (5 g of dry broccoli) was 10.5±0.8 µmol of GI and 70±2.7 µmol of GR. MYR activity in fresh broccoli (High MYR BF and High MYR BP) was 6.41±0.8 U/g DW. MYR activity of Medium MYR BF, Low MYR BF and No MYR BF and the different processing conditions are reported in the Table 6.1.

MYR activity and SR and IB formation in the rehydrated broccoli products

In order to retain GR, GI and MYR, mild rehydration conditions for the dried broccoli were selected. The rehydration of the broccoli products in 90 mL of water at 40 ˚C for 2 min, did not change MYR activity ($p > 0.05$). SR and IB content of the rehydrated broccoli products are shown in Table 6.1. The highest SR and IB formation can be seen in the High MYR BP. The High MYR BP resulted is a double IB and SR formation than the High MYR BF product. Besides, in all the products, the percentage of GI hydrolysis extent was higher than the percentage of GR hydrolysis.

SR and IB conjugates excretion in urine

The cumulative amounts of SR and IB conjugates excreted in 24 h urine after consuming each rehydrated broccoli product are reported in the Table 6.1. The higher the MYR and SR and IB formation in the rehydrated broccoli products, the higher was the cumulative amount of SR and IB conjugates in urine.

The bioavailability of SR and IB, calculated as cumulative μmol of SR and IB conjugates excreted in 24 h urine divided by the μmol GR and GI occurring in the broccoli products, is reported in Table 6.2. It represents the fraction of SR and IB formed in the rehydrated broccoli products that reaches the systemic circulation and in addition the fraction of SR and IB formed by the MYR activity of the enteric microflora. It means that the bioavailability of SR and IB after consuming No MYR BF, exclusively represents SR and IB formation extent by the MYR activity of the enteric microflora. The higher the SR and IB formation in the rehydrated broccoli products, the higher SR and IB bioavailability. In particular the bioavailability of SR and IB after consumption of High MYR BP was higher than after consumption of No MYR BF (around 83% and 79% respectively). Moreover, the bioavailability of IB was around 38% higher than SR bioavailability among all broccoli products. Besides, the GI hydrolysis extent in the gut was 47% higher than the GR hydrolysis after consumption of No MYR BF.

The typical kinetics of SL and IB conjugates excretion curves after consumption of the 5 broccoli products are shown in Figure 6.1. Depending on the consumed broccoli product, five different excretion curves can be noticed. High MYR BP, High MYR BF and Medium MYR BF curves show similar shape due to the max excretion peak that appears within 3 h after consumption (Table 6.2 and Figure 6.1). Low MYR BF and No MYR BF curves have also similar shape due to the max excretion peak that appears within 4-6 h after consumption (Table 6.2 and Figure 6.1).

Table 6.1: Description of 5 broccoli products: processing conditions, myrosinase (MYR) residual activity, glucoiberin (GI), glucoraphanin (GR), iberin (IB) and sulforaphane (SR) profiles. Cumulative IB and SR conjugates excreted in 24 h urine after consumption each rehydrated broccoli product (n=15).

Broccoli products	Microwave treatment conditions before freeze drying	MYR activity in 5 g of dried broccoli portions (Units± SD)	GI and GR content in 5 g of dried broccoli portions (µmol± SD)		IB and SR content in the rehydrated broccoli product (5 g dried broccoli and 90 mL water, µmol± SD)		Cumulativ IB and SR conjugates excreted in 24 h urine (µmol)	
			GI	GR	IB	SR	IB conjugates	SR conjugates
High MYR BP	None	31 ± 4	11 ± 3.8	73 ± 4.6	10.1 ± 0.1	34.0 ± 0.8	8.8 ± 2.5^{a}	40.4 ± 10.4^{a}
High MYR BF	None	31 ± 4	11 ± 3.8	73 ± 4.6	6.8 ± 0.1	11.1 ± 0.6	4.6 ± 1.3^{b}	23.1 ± 8.9^{b}
Medium MYR BF	180 W, 8 min	6 ±1	10 ± 1.1	69 ± 6.7	6.2 ± 0.3	6.7 ± 1.9	3.7 ± 1.2^{bc}	15.6 ± 3.7^{bc}
Low MYR BF	540 W, 3 min	0.5 ± 0.1	9.8 ± 0.3	67 ± 3.9	3.4 ± 1.8	2.8 ± 1.8	2.9 ± 0.9^{c}	11.7 ± 4.1^{c}
No MYR BF	900 W, 5 min	No detectable	9 ± 1.1	72 ± 4.5	0.2 ± 0.1	0.3 ± 0.2	1.9 ± 1.0^{d}	7.1 ± 2.9^{d}

Different letters within a column indicate significant differences ($p < 0.05$).

Table 6.2: Urinary pharmacokinetics. Iberin (IB) and sulforaphane (SR) conjugates excretion after consumption of 5 g of dried broccoli (n=15).

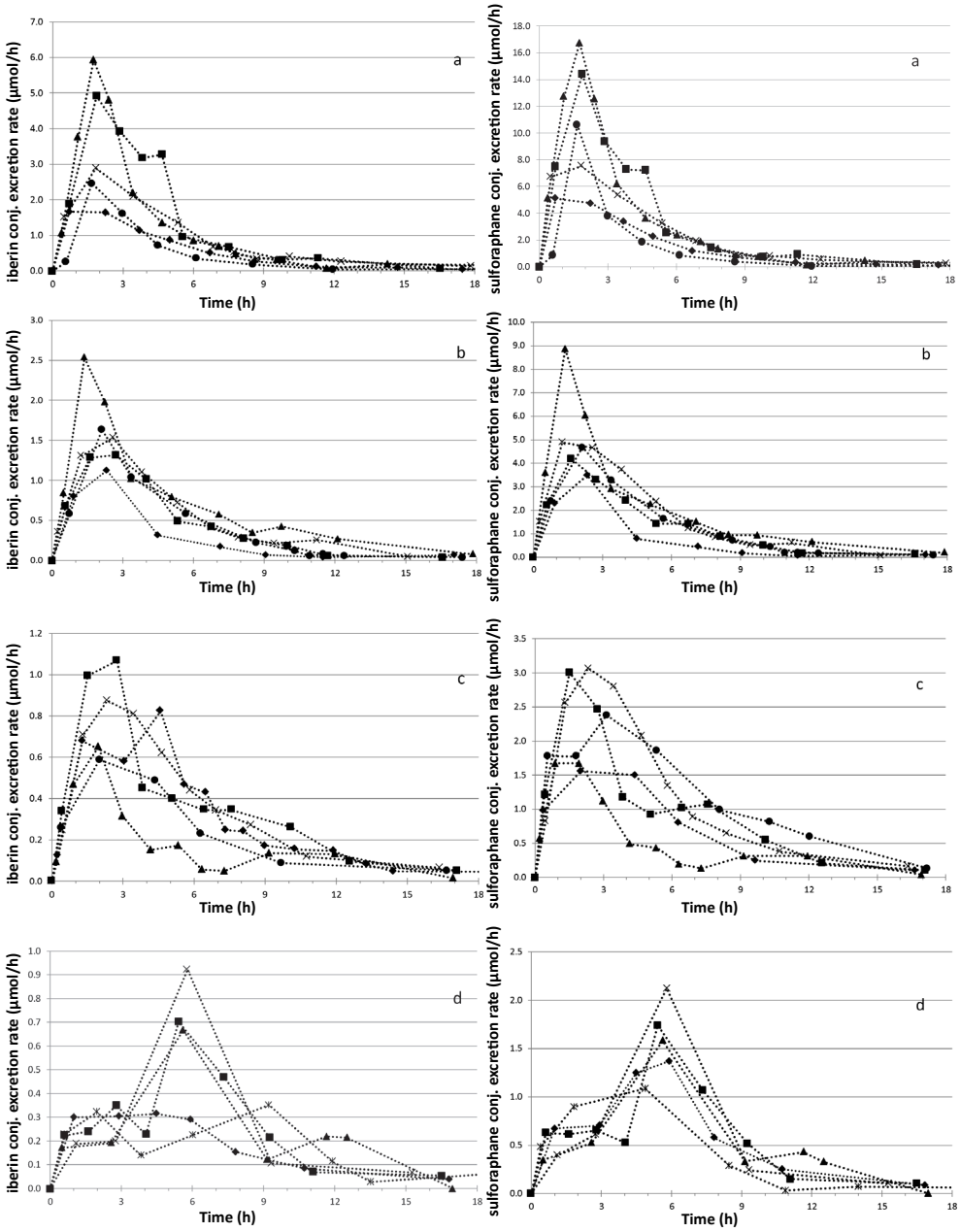
Broccoli products	Bioavailability (%)		peak time (t_{max}, h) (± SD)		Excretion half-life ($t_{1/2}$, h)	
	IB	SR	IB conjugates	SR conjugates	IB conjugates	SR conjugates
High MYR BP	88 ± 24^{a}	58 ± 14^{a}	2.3 ± 0.8^{a}	2.3 ± 0.8^{a}	2.1 ± 1.0^{a}	1.9 ± 0.9^{a}
High MYR BF	46 ± 13^{b}	33 ± 12^{b}	3.4 ± 1.5^{ad}	3.1 ± 1.5^{ad}	2.2 ± 0.8^{a}	2.2 ± 0.8^{a}
Medium MYR BF	37 ± 11^{bc}	22 ± 4.8^{bc}	3.5 ± 2.0^{ab}	3.4 ± 2.1^{ab}	2.8 ± 1.4^{a}	2.7 ± 1.4^{a}
Low MYR BF	29 ± 8.9^{c}	17± 4.8^{c}	4.7 ± 2.3^{bd}	4.5 ± 2.1^{bd}	2.8 ± 1.4^{a}	3.1 ± 1.0^{a}
No MYR BF	19 ± 10^{d}	10 ± 3.8^{d}	6.1 ± 1.9^{b}	6.1 ± 1.9^{b}	2.8 ± 1.1^{a}	2.5 ± 0.9^{a}

Different letters within a column indicate significant differences ($p < 0.05$).

Discussion

GR, GI content and MYR in dry broccoli products

Microwave treatments were meant to decrease, at different extent, MYR activity while retaining GLs. The applied microwave treatments and the following freeze drying did not significantly change the GI and GR content. As expected MYR activity changed drastically after microwaving (Table 6.1). In agreement with Verkerk *et al.* 2004, no detectable MYR activity in No MYR BF after microwaving at 900 W for 5 min was found. In our study 80% MYR activity reduction was found after microwaving at 180 W for 8 min, whereas Verkerk *et al.* (2004) could not find MYR reduction by microwaving red cabbage at 180 W for 12 min. In our study 98% MYR activity reduction was found after microwaving 540 W for 3 min whereas 60% reduction was found after microwaving red cabbage at 540 W for 4 min (Verkerk *et al.,* 2004). MYR stability during heat treatments can be very different depending on the vegetables and also on the heating condition, such as vegetable chopping size, amount of cooking water, etc. (Verkerk *et al.*, 2004).


iberin conj. excretion rate (µmol/h)
sulforaphane conj. excretion rate (µmol/h)
Time (h)
a
b
c
d

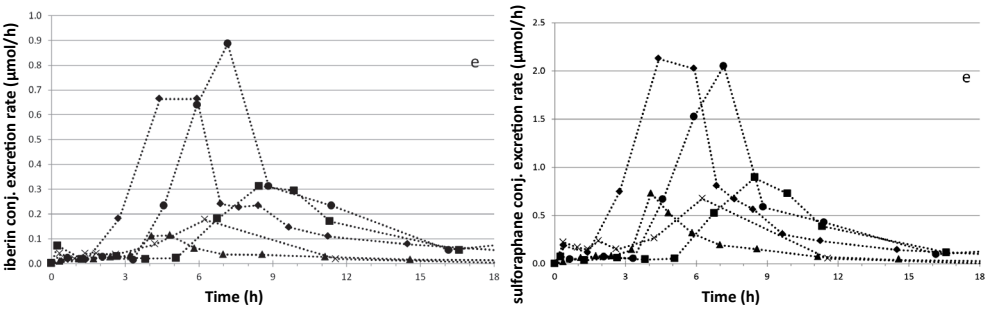


Figure 6.1: Five typical sulforaphane and iberin conjugates excretion curves selected among 15 participants (the highest, the lowest and the intermediate excretion curves for each product) after consumption of: a) High myrosinase broccoli powder (High MYR BP); b) High myrosinase broccoli florets (High MYR BF); c) Medium myrosinase broccoli florets (Medium MYR BF); d) Low myrosinase broccoli florets (Low MYR BF); e) No myrosinase broccoli florets (No MYR BF).

MYR activity and SR and IB formation in rehydrated broccoli products

The broccoli rehydration condition did not affect MYR activity. Such mild condition was selected to retain MYR activity of dried broccoli. No MYR reduction was shown also by heating broccoli at 50 ˚C for 2 min (Van Eylen *et al.,* 2008). After rehydration SR and IB were formed due to MYR activity of the samples and the highest formation can been seen in High MYR BP. Despite having the same MYR activity and GI and GR initial concentration, in High MYR BP the formed IB and SR was twice the amount formed in High MYR BF. The lower SR and IB formation in the florets products can be explained by the intact structure of the florets that prevented MYR to hydrolase GR and GI during 2 min rehydration. As expected in the florets products the higher the MYR activity, the higher SR and IB formation were formed. The freeze drying may have partially damaged the broccoli cell structure, allowing part of the MYR to hydrolyse GR and GI during rehydration. It is interesting to notice that under the same rehydration conditions the percentage of GI hydrolysis was higher than the percentage of GR hydrolysis (Table 6.1). For instance, in the High MYR BP rehydrated broccoli product 91% of IB and 48 % of SR (as proportion of the corresponding GL in dried broccoli) was formed whereas in High MYR BF, 62 % of IB and 16 % SR was formed. Very little literature is available on different catalytic efficiency of MYR. It has been reported that aliphatic GLs are hydrolysed faster than indolic GLs (Francis *et al.*, 2002). MYR extracted from cabbage aphid showed higher affinity for sinigrin (allyl glucosinolate) than for glucotropaeolin (benzyl glucosinolate) (Jones *et al.,* 2001).

SR and IB conjugates excretion in urine

In this study, GLs and ITCs were ingested and ITC conjugates in urine reflect the hydrolysis, absorption, metabolic conversion and excretion as also shown by (Shapiro *et al.* 1998; Shapiro *et al.,* 2001). A high standard deviation was found in the cumulative amount of SR and IB conjugates after consuming the five broccoli products, this variability may be due to variations related to the participants of the study by: (1) Different intake of SR and IB formed in the rehydrated broccoli products, *i.e.*, different proportion of SR and IB pre-formed in the rehydrated broccoli products ingested by participants, because of different eating time and different chewing extent among participants. (2) Difference in enteric microflora MYR-like activity among the participants and other kind of products formed by GLs hydrolysis in the gut (Combourieu *et al.,* 2001). (3) Other metabolic paths such as the inter-conversion of SR in erucin (Clarke *et al.*, 2011).

The cumulative SR and IB conjugates excretion in urine was positively associated to the amount of residual MYR activity and pre-formed SR and IB in the rehydrated broccoli products (Table 6.1). The cumulative SR and IB conjugates excretion in urine was the highest after ingestion of High MYR BP and the lowest after ingestion of No MYR BP. The cumulative SR and IB conjugates excretion was lower in High MYR BF compared to High MYR BP ($p < 0.05$) due to the higher SR and IB pre-formed in the High MYR BF. These results are in line with other *in vivo* studies, where the excretion of ITCs is investigated in *Brassica* vegetables with and without MYR, in which it is shown that ITCs conjugates excretion is higher after ingestion of *Brassica* vegetables with active MYR or with higher ITCs (Shapiro *et al.,* 2001; Rungapamestry *et al.,* 2007; Vermeulen *et al.*, 2008). Nevertheless, in our study, after No MYR BF rehydrated broccoli product ingestion, that contained 0.2 ± 0.1 and 0.3 ± 0.2 µmol of IB and SR, 1.9 ± 1.0 and 7.1 ± 2.9 µmol of IB and SR conjugates were excreted in urine (Table 6.1). This ITCs formation can be ascribed to GR and GI hydrolysis catalysed by the intestinal microflora (Shapiro *et al.,* 2001; Rungapamestry *et al.,* 2007; Vermeulen *et al.,* 2008). The cumulative SR conjugates in urine excreted was higher than the amount pre-formed in the rehydrated broccoli products, that would indicate that SR formed during chewing contributed highly to its final bioavailability. It is interesting to notice that the bioavailability of IB is higher than the bioavailability of SR in all the tested products, even if IB and SR differ of only one methyl group on the side chain. Different ITCs bioavailability, between 50 % and 113 % (calculated as cumulative ITCs conjugated in urine divided by the ITCs found in the vegetables), was also found after ingestion of different raw *Brassica* vegetables

containing different ITCs (Vermeulen *et al.,* 2006). It is also interesting to notice that the extent of GI hydrolysis, catalysed by the MYR-like activity of enteric microflora, was 2-fold higher than the GR hydrolysis extent (Table 6.2). Different hydrolysis extent by the enteric microflora, among different GLs present in one *Brassica* cultivar is also reported in literature (Vermeulen *et al.,* 2006). For instance, the hydrolysis extent (or bioavailability calculated as cumulative ITCs conjugated in urine divided by the GLs in the vegetable) of GR and sinigrin after ingesting cooked Brussels sprouts, was 5.2% ± 2.3 and 9.3% ± 5.8 (Vermeulen *et al.,* 2006).

The bioavailability results (Table 6.2) show that even if High MYR BP and High MYR BP had similar GLs and MYR, due to the grinding step after drying, more ITCs were formed in High MYR BP than in High MYR BF during rehydration, leading to higher ITCs intake. The Medium MYR BF product showed similar bioavailability values than the High MYR BF product ($p > 0.05$), suggesting that even if MYR was reduced by 80% during processing, the ITCs bioavailability was as high as after consuming 1/1 MYR broccoli florets (High MYR BF). The Low MYR BF product had a significantly lower (63% for IB and 51%, for SR) bioavailability compared to the high MYR BF, but showed significantly higher (34% and 41% IB and SR) bioavailability values than the No MYR BF ($p < 0.05$), suggesting that even a low MYR activity may lead to a higher bioavailability that No MYR BF. All in all, the results indicate clearly that the bioavailability is highly related to MYR activity of the tested products.

The excretion kinetics of IB and SR appear very similar to each other after ingesting the five products (Figure 6.1 and 6.2). The max excretion peaks of SR and IB conjugates after ingestion of No MYR BF and Low MYR BF appeared 1-3 h later than after ingestion of the rehydrated broccoli product with high IB and SR content and high MYR activity (High MYR BP, High MYR BF and Medium MYR BF). Such different ITCs conjugates max excretion peaks appearance have been reported after consuming raw and cooked *Brassica* vegetables in urine and in blood (Rouzaud *et al*., 2004; Shapiro *et al.*, 2001; Vermeulen *et al.*, 2008). In these studies the MYR of the raw and cooked products was not analysed, but it can be assumed that their cooking methods had completely inactivated MYR. The delay in the ITCs conjugates max excretion peaks, after consuming cooked broccoli (inactivated MYR) can been ascribed to later dietary GLs hydrolysis that occurred in the intestinal tract catalysed by the enteric microflora (Conaway *et al.*, 2000; Vermeulen *et al.*, 2008). Although the max peak time clearly

depends on the MYR in the products, no difference in excretion half-life ($t_{1/2}$, h) of ITCs conjugates among the products, was shown. It suggests that after the max excretion peaks appear, ITCs conjugates elimination was similar regardless the amount of ITC absorbed. Similar half-life ITC conjugates excretion were found after ingesting of many *Brassica* vegetables and no difference was found between raw and cooked *Brassica* (Vermeulen *et al.*, 2006; Vermeulen *et al.*, 2008). Although the metabolism and bioavailability of ITCs have been well studied, little is known about the effect of ITCs concentration, ITCs excretion half-life and maximum excretion peak time on ITCs anticarcinogenic efficiency. It is unclear whether a higher and sudden ITCs release in the body would have a better anticarcinogenic effect than a slower and gradual release. On one hand, the epidemiological studies that suggest the inverse association between consumption of *Brassica* vegetables and the risk of many types of cancer (Traka & Mithen, 2009) will be mainly based on the consumption of cooked vegetables, as they are the primary source of *Brassica*'s in the diet, suggesting that slower and gradual release of ITCs may be enough to obtain a protective effect against cancer. On the other hand, it is not known if a higher and sudden ITCs release, resulting from raw and semi-raw *Brassica* vegetables consumption, in the body may be beneficial for the protective effect of *Brassica* consumption towards cancer. A study suggests that temporary or continuous exposure of human colon carcinoma cells to SR may have a different inhibition effect on these cells cycle (Pappa *et al.,* 2007). Further studies are needed to clarify the cell cycle arrest mechanism caused by SR, related to the kinetics of ITC bioavailability upon *Brassica* vegetables consumption.

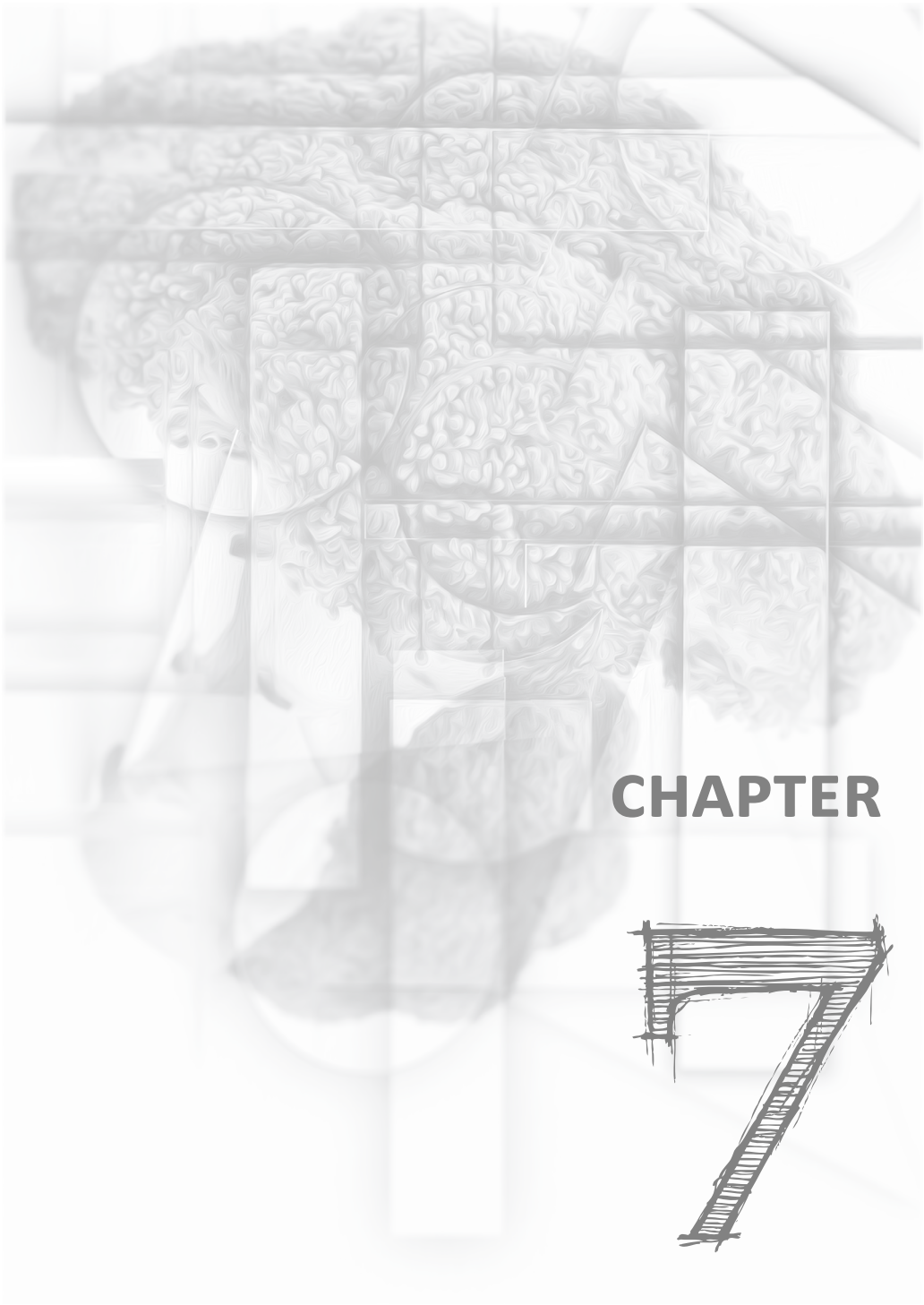
The bio-formation, bioavailability and excretion kinetics after consumption of differently microwaved and then freeze dried and rehydrated broccoli products with the same GLs content and different MYR activity, were systematically investigated. For the first time it was shown that the occurrence of a residual, although small, MYR activity led to a significantly higher ITCs bioavailability than after consumption of broccoli with no residual MYR activity. The bioavailability of ITCs after consuming broccoli where MYR was reduced by 80 % (due to microwave treatment), was not significantly different from consuming raw-like broccoli with 100 % MYR activity. This is a very interesting result because broccoli is recommended to be cooked before consumption, as it contains irritants that can cause bloating, gas and abdominal cramping (Shahidi *et al.*, 1990). Moreover, the bioavailability of ITCs after consuming powdered raw-like broccoli (100 % MYR activity) was higher than after consuming raw-like broccoli florets (100% MYR

activity), due to higher ITCs formation in powdered broccoli. This finding shows that to enhance ITC uptake, from broccoli-based dried products, a powdering process after drying is advisable to obtain a product that lead to high ITCs uptake. Food processing can reduce the healthiness of vegetable consumption, but the use of controlled processing to achieve products with specific characteristics, having specific health effect, would be beneficial for consumers. This study suggests that food processes can be designed to produce products with specific bioactive compounds profiles.

Acknowledgement

This work is supported by the Energy Research Program EOS of the Dutch ministry of Economics under project EOSLTe7043.

General discussion



CHAPTER

7

Introduction

Food safety, palatability and digestibility issues require the use of food processing for transformation of raw food. Processing of food induces several biological, physical and chemical modifications, leading to safety, sensory and nutritional changes. Industrial food processing is often negatively perceived by consumers as treatments that lead to the formation of harmful compounds, or to the degradation of healthy compounds. Although certain reactions under some process conditions may indeed lead to the loss of nutritional value and the formation of potentially toxic compounds, many other reactions result in safer foods, the formation of desired flavours and an increase in the bioavailability of healthy compounds (Van Boekel *et al.* 2010). Air drying is one of the technologies that most affect the food organoleptic properties, altering completely food appearance, texture and reducing the food nutritional value if not controlled properly. Glucosinolates (GLs) concentration and myrosinase (MYR) activity can be drastically reduced by food processing like air drying. Nevertheless, air drying is a useful and widely used process to increase storage stability and facilitate transport efficiency. Hence, there is a need to design air drying processes which retain most of GLs and MYR activity.

The first aim of the present research was to kinetically model the effect of temperature and water concentration/activity on GLs degradation and MYR inactivation in broccoli (*Brassica oleracea* var*. italica*) (**Chapter 3** and **4**). These models were used to optimize air drying conditions to obtain broccoli products with certain GLs levels and MYR activity (**Chapter 5**). The second aim was to investigate the kinetics of absorption of ITCs in human body after consuming broccoli products with different GLs, MYR and ITCs profiles (**Chapter 6**). The concept of the thesis is explained in Figure 7.1.

This discussion starts with a summary of the main findings, followed by methodological considerations and the discussion and interpretation of the results. Finally, future prospects and main conclusions of the research are described.

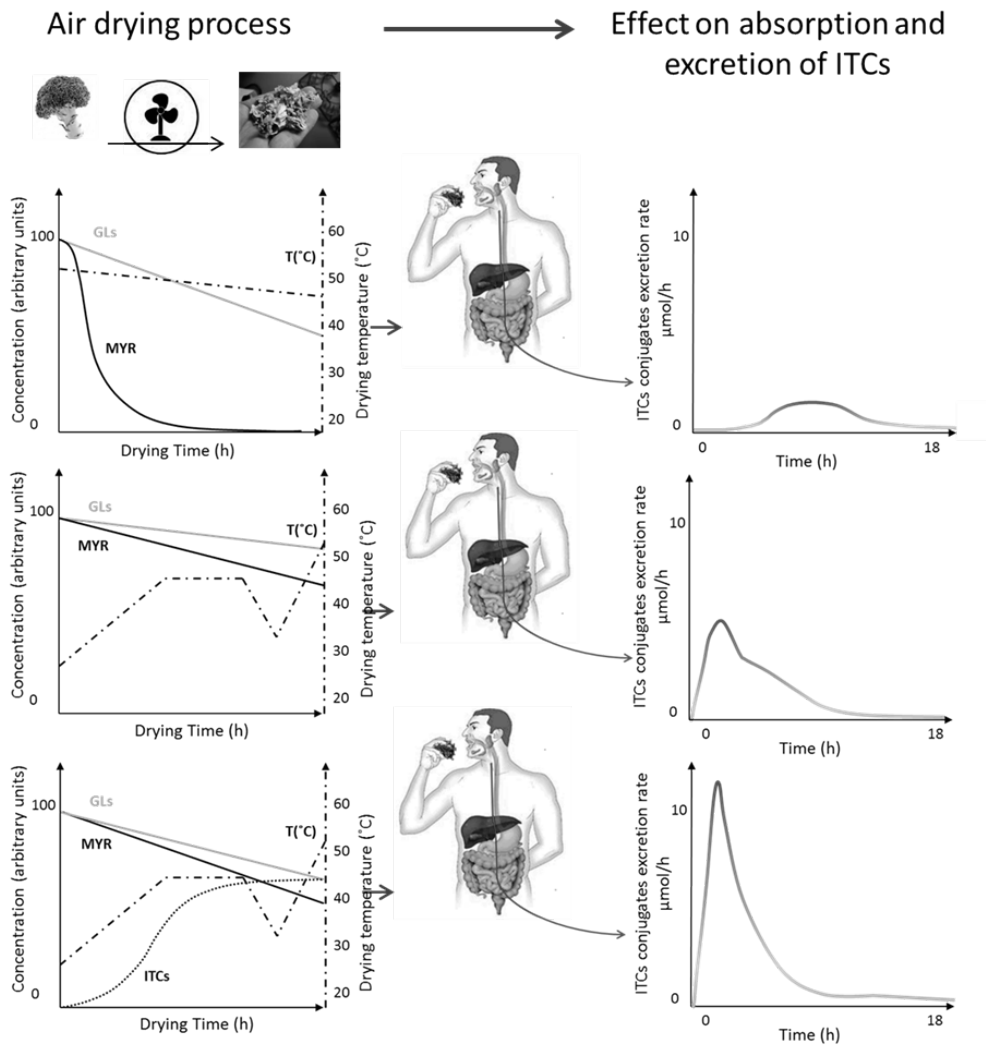


Figure 7.1: Schematic illustration of the thesis concept

Main findings

In order to optimize air drying process conditions to produce dried broccoli with the highest retention of GLs and MYR, thermal degradation and inactivation kinetics of GLs and MYR were studied. During drying, broccoli is subject to changes in water content and temperature which affects the concentration of GLs and MYR activity. Hence, the effect of water content and temperature on GLs degradation and MYR inactivation kinetics was studied (**Chapter 3, 4**). Batches of broccoli with different water content were incubated for various times at different temperatures and GLs concentration was monitored (**Chapter 3**). GLs degradation could be described by first-order kinetics. GLs degradation rate constants increased with temperature for all water content investigated. Water content was found to have a remarkable effect on GLs degradation rate constants. The lowest degradation rate constants were found in the driest broccoli (13% water content) at temperature ≤ 100°C and an increase in degradation rate constants was observed as the water content increased (from 34 up to 82%). At 120°C the inverse was observed with the driest broccoli showing the highest GLs degradation rate constant. To study MYR inactivation, batches of broccoli with different water content were incubated for various times at different temperatures and MYR activity was monitored (**Chapter 4**). MYR inactivation could be described by a two consecutive steps reaction model. MYR inactivation rate constants also increased with temperature for all water content investigated. Also for MYR, the water content had a large effect on the inactivation rate constants. MYR inactivation rate constants were lower in the driest samples (10% water content) at all the studied temperatures. Broccoli with 31%, 67% and 90% water content showed similar inactivation rate constants.

Then, the kinetic parameters of glucoraphanin (GR) degradation and MYR inactivation estimated in the previous studies, along with kinetic parameters for Vitamin C degradation taken from literature, were used to optimise air drying conditions (**Chapter 5**). Dynamic optimization was applied to find the temperature trajectories that minimize GR and Vitamin C degradation and MYR inactivation. The obtained optimized temperature trajectories were also experimentally tested to monitor GR and MYR activity and vitamin C. Simulation and experimental results for optimized temperature trajectories were compared to constant inlet air drying temperature at 40 and 50°C. The optimized temperature trajectories led to

remarkable improvement of the retention of GR, vitamin C and MYR, but tended to overestimate vitamin C and GR degradation and MYR inactivation.

In **Chapter 6** a human intervention study was performed to investigate *in vivo* the effect of broccoli products with different GLs, MYR and ITC profiles. Broccoli batches were differently processed in order to obtain 5 dried products with different GLs, MYR and ITC profiles. These products were consumed by 15 human volunteers in a cross-over design study. GR, glucoiberin (one of the GLs present in broccoli) and their ITCs, sulforaphane (SR) and iberin (IB), were determined in the broccoli products and their excreted metabolites in urine (ITCs conjugates). In particular, the role of different MYR activity in the broccoli products on ITC formation, bioavailability and excretion kinetics was investigated. The first broccoli product had high concentrations of pre-formed SR and IB. In the other four broccoli products the GR and GI concentration was similar, whereas residual MYR activity was high (100%), medium (20%), low (2%) and inactivated. The broccoli product with 20% MYR activity showed similar bioavailability (calculated as cumulative µmol of SR and IB conjugates excreted in 24 h urine divided by the µmol of GR and GI occurring in the broccoli products) as the broccoli product with high MYR activity. The MYR broccoli product with low residual MYR activity showed less bioavailability compared to the products with high and medium residual MYR activity, but still significantly higher bioavailability values than the broccoli product with no residual MYR activity. In addition, the maximum excretion peaks of SR and IB conjugates after ingestion of broccoli with no or low residual MYR activity, appeared 1-3 h later than after ingestion of broccoli products with intact MYR activity.

Methodological consideration

Kinetic modelling to design a food process

The dynamic optimization of air drying temperature trajectory was based on the kinetics of GR thermal degradation and MYR inactivation in broccoli with different water content. In order to rule out experimental variables that could interfere with the interpretation of the results, these kinetic experiments on broccoli were performed by using a simplified system. For both GLs degradation and MYR

inactivation kinetic studies (the two experiments were performed separately), batches of broccoli with different water content were produced by freeze drying. The advantage of using freeze drying was to prevent significant reduction of GLs and MYR activity. A microwave treatment was applied before freeze drying, only in the GLs study. The aim of the microwaving was to inactivate MYR while retaining GLs (Verkerk & Dekker, 2004; Oliviero *et al.*, 2012), to rule out the enzymatic hydrolysis of GLs and isolate the effect of thermal treatment on GLs degradation. In both the studies, after freeze drying, broccoli florets were ground (by using a blender and liquid nitrogen) and kept frozen till heating. The thermal treatments were performed in hermetic metal tubes with an inner thermocouple placed in a heating block, to monitor the temperature profile. The kinetics of GLs degradation (in particular of GR) and MYR inactivation, along with kinetics of Vitamin C (Vc) degradation obtained from literature (Villota & Karel, 1980), were used for the dynamic optimization of air drying temperature trajectory. Apart from a higher retention of Vc, GL and MYR achieved by the optimized drying temperature trajectory, another outcome was that the simulation results at constant temperature and at temperature trajectory, tended to overestimate the GR and Vc degradation and MYR inactivation. For Vc this discrepancy could be due to the fact that a model and parameters were taken from literature based on isolated Vc and not a food (broccoli) matrix. For MYR the overestimation of residual activity could be due to the fact that in the optimisation a first order model was used, while in fact a consecutive model describes the inactivation better, leading to a more stable enzyme fraction especially for the driest products. In addition, these discrepancies could have been caused by to the different way the heating treatments were performed. The simulation was carried out by using kinetic data obtained by heating homogenised broccoli tissue in a simplified system, *i.e.* metal tubes in heating block. Conversely, to test the optimization trajectory and constant temperature drying, intact broccoli florets were dried in a pilot air dryer. The simplified heating system, advantageous to rule out the interference of unwanted variables and to gain basic knowledge on GLs and MYR inactivation, showed here its limitation because it did not take into account other variables that may affect the components during air drying of a real food.

Human intervention issues

In vitro studies allow researchers to investigate the bioactivity of molecules in controlled environment outside of a living organism. Such studies are performed either on component of a living organism, *e.g.* in cells, or in systems that mimic a component of an organism, *e.g. in vitro* gastrointestinal tract model. The weaknesses of *in vitro* experiments are the challenge in translating the results to the biology of an intact living organism. To have a complete picture, an *in vitro* study can be followed by an *in vivo* study. The *in vivo* studies are performed on animals or humans (human intervention studies), testing the bioactivity of molecules in a whole living organism. *In vivo* studies allow to observe the overall effects of an experiment on a living subject and can offer conclusive insights about the effect of the target molecule. However, there is a number of ways that these conclusions can be misleading.

To reduce the chance of obtaining misleading results, a proper experimental design has to be prepared to obtain significant results that can answer the research questions. After selecting the analytical methods to detect the targets compounds (both as present in the food or pill etc. and as absorbed in the body) it is recommendable to run a pilot experiment mainly to find out the variation of the data and also to find out the possible pitfalls of those methods, to better design the experiment. In this study, a pilot experiment was performed before the human intervention study. It was found very useful to know variation in the variable investigated and to select the appropriate statistical test to calculate the sample size to be able to detect a significant difference, if any. The pilot experiment also gave insight on how practically organize the eating sessions, in collecting and delivery urine samples and the subsequence urine analysis.

To reduce the chance of obtaining misleading results, it is also important the communication with the participants of the study. After recruiting the participants, it is crucial to give to each participant an informative booklet to explain their duties and rights and a questionnaire to be able to know information that otherwise participants will not consider important to report to the researcher.

Discussion and interpretation of the results

In order to optimize air drying process conditions to produce dried broccoli with the highest retention of GLs and MYR, a study on thermal degradation and inactivation kinetics of GLs and MYR was performed. During drying, broccoli is subjected to changes in water content and temperature which affect the concentration of GLs and MYR activity.

Water content can affect mechanical and physical properties of food, influencing the rate constants of reactions in foods. Water can act as (1) reagent, (2) solvent and catalyst (Oliver *et al.,* 1998). (1) When water acts as reagent, as in the case of hydrolysis reaction, it directly affects the reaction rate by its chemical activity. The reaction rate will increase with increase of a_w (to a certain extent) and in this case only very low a_w may have a limiting effect on the reaction. (2) Water can also act as solvent affecting the mobility and the concentration of reagents. The diffusion of reagents can be hindered by the smaller pore size and tortuosity in foods with a very high solid contents. As a result, the effective diffusion coefficient becomes smaller, decreasing the reaction rate (Van Boekel, 2009), although the dependence of diffusion coefficient from a_w is not linear. Food with low a_w can also be in a glassy state. In general, a glassy state refers to amorphous solids with very high viscosity. Food can (partly) be in a glassy state when water is removed to a large enough extent, *e.g.* upon drying, extrusion, freezing etc. The glassy state is characterized by a very low molecular mobility, hindering reactions to occur, reducing the overall rate of reactions. Indeed, low a_w food have long shelf life. On the other hand, by removing water, the concentration of reagents increase, increasing the rate of reaction (to a certain extent).

The results on the kinetic of GLs degradation in broccoli showed that the lowest degradation rate constants were found in the driest broccoli (13% water content) at temperature ≤ 100°C The GLs degradation is a hydrolysis reaction (no mechanism alternative to hydrolysis has been reported so far) therefore water acted as reagent, and at temperatures ≤ 100°C the low a_w of the driest broccoli limited the reaction rate. An increase in degradation rate constants was observed as the water content decreased in broccoli with water content ranged from 34 up to 82%. (**Chapter 3**). This effect of water on reaction rate constants may be explained by the change of the activity coefficient of reagents. According to the transition state theory, the

rate of a reaction in non-ideal solution, such as broccoli, depends on the activity coefficients of reactants and that of the activated complex. In non-ideal conditions the following relation holds for a bimolecular reaction:

$$k_{obs} = k_{id}\left(\frac{\gamma_{GL} * \gamma_{H2O}}{\gamma ‡}\right)$$

Where k_{obs} is the observed rate, k_{id} is the kinetic rate in ideal conditions and γ_{GL},γ_{H2O} and γ‡ are the activity coefficients for GL, H_2O and the transition state respectively. If the activity coefficients change in a way that the ratio (γ_{GL}*γ_{H2O}/γ‡) increases, the rate of the reaction increases. The activity coefficients of GLs are expected to increase as the a_w decreases because of the volume exclusion effect on GLs free energy. If the increase in the activity coefficient of GLs is high enough and such that the ratio (γ_{GL}*γ_{H2O}/γ‡) to be >1, the rate of the hydrolysis reaction increases despite the decrease of the activity coefficient of water, explaining the order of degradation rates of GLs observed at temperatures ≤ 100°C. The activity coefficients concept does not explain the lowest degradation rate of the sample with 13% water content. In fact, at 120°C the driest broccoli (13% water content) showed the highest GLs degradation rate constant. An explanation for this could be that in such a sample the very low a_w would decrease the molecular mobility of the reactants. The GLs hydrolysis reaction would switch from reaction-limited to diffusion-limited in the conditions (Van Boekel, 2009). At 120°C, as the molecular mobility increases, the reaction rate would be less diffusion-limited. The a_w at 25 ˚C (the temperature at which the a_w of the broccoli was analysed) of broccoli with 13% water content, was 0.32 and at 120 ˚C was found 0.47 (calculated from the Clausius-Clapeyron equation). Hence, the a_w of the driest samples slightly increased at 120 ˚C, but it did not reach the a_w levels of the broccoli with higher water content, but it may have contributed to the increase the degradation rate constants. It is likely that both these effects, namely that of a_w on activities and that on mobility of reactants contribute to the effect of WC on the degradation rates of GLs in dried broccoli.

To compare with other results in literature, also the degradation rate constants of chlorophyll in Yerba maté leaves were reported to be effected by water content. The rate constant of chlorophyll (at 50-70˚C) decreased with the decrease of water activity, while this effect became less evident with increasing temperature (80˚C)

(Schmalko *et al.*, 2007). These results may be explained by the diffusion-limitations in the low water content sample. Also for vitamin c in guava, a decrease of rate constants with decrease in water activity is reported (Uddin *et al.*, 2002). However, examples of not linear relation between a_w and degradation rate constants are also showed in literature. For instance, carotenoids in carrots shoved lowest degradation constants at a_w range of 0.31-0.54 at temperature tested of 40°C (Lavelli *et al.*, 2007). MYR thermal inactivation was affected by the water content as well. The results showed that the inactivation rates constants were similar among broccoli with water content between 31-90%, whereas the driest broccoli showed the lowest inactivation rate constants at all the temperatures studied (**Chapter 4**). This stability may be explained by low conformational mobility of MYR in low water content broccoli. In a molecular crowded system, as a low water content system, there may be no sufficient volume for an enzyme to unfold so that it will not lose its activity. The inactivation rate of peroxidase enzyme was found to be lower in solid state (low water activity) than in solution (Hendrickx *et al.*, 1992).

The mathematical simulation of drying at both the optimized temperature trajectories and at constant temperature tended to overestimate the GR and Vc degradation and MYR inactivation (**Chapter 5**). The kinetics of Vc degradation, obtained from literature, were studied in a model system that consisted of inert material in which Vc was added. Model systems are often diluted systems used to study the kinetics of reactions. The purpose of studying a reaction in such system where only the reagents are present is to understand the mechanism of that reaction. However, information obtained by studying a reaction in a model system may not be directly translated to real systems. Food matrix, as real system, has a large impact of the kinetic of a reaction. The possible effects of food matrix on reaction kinetics are various. Among them, molecular crowding or volume exclusion, *i.e.* the occurrence of the other compounds, especially high molecular weight compounds that affect the reaction rate (**Chapter 3**); buffering effect of some compounds and presence of ionic or nonionic solutes; occurrence of lipids, emulsions, foam and surfactants. In this thesis the kinetic of GLs degradation and MYR inactivation were studied in real broccoli, to be able to apply the information on kinetics to real air drying and to obtain reliable simulations. However, the MYR inactivation and GR degradation kinetics were performed in broccoli, but not on intact broccoli florets as for the air drying experiments, due to practical reasons. The plant matrix of intact florets may

have affected the GLs degradation and MYR inactivation. In intact florets GLs are localised in the vacuoles and MYR is localized in special vacuole-like myrosin cells, whereas upon grinding this structure is lost (Kissen *et al.*, 2009). The release of the compounds may have enhanced their degradation or inactivation, whereas the plant structure may have had a protective effect on GR and MYR. The different heat treatments may also have affected GLs and Vc degradation and MYR inactivation. During drying water evaporates from the surface of broccoli and from the inside to the outside of broccoli. As a result, the concentrations of reactants increase and at the same time a concentration gradient arises. In addition, in intact broccoli also transport due to the capillary action may occur. These changes in concentration will influence the reaction kinetics. In the simplified heating system the heating tubes were closed so all the transport phenomena and the changes in concentration were reduced.

Further research should be performed to investigate the effect of the complexity of food matrix on reaction kinetics to better predict the formation and the degradation of compounds. The use of model system to investigate the kinetics of a reaction is a useful tool to better understand the reaction mechanisms, but it should be performed along with validation experiments to test the reaction kinetics in real food.

A human intervention study was performed to investigate *in vivo* the effect of broccoli products with different GLs, MYR and ITC profiles. Because of the GLs hydrolysis, only a certain amount of GLs and converted in ITCs, the quantification of GLs and MYR activity in vegetable is not an accurate measure of the actual intake of ITCs in humans. The quantification of ITCs conjugates excreted in urine after *Brassica* vegetables consumption gives a better idea on the extent of ITCs formation and absorption by the human body (Shapiro *et al.*, 1998; Vermeulen *et al.*, 2008). The results of this study suggest that even low residual MYR activities in the broccoli product are sufficient to hydrolyse glucoraphanin and glucoiberin during mastication, highly contributing to the formation of their ITCs, sulforaphanin and iberin, and therefore to their final bioavailability. The excretion of sulforaphanin and iberin conjugates in urines after consumption of the product with no residual MYR activity confirmed that glucoraphanin and glucoiberin can be hydrolysed in the human gut by the MYR-like activity of human enteric microflora (Shapiro *et al.*, 1998), though at a much lower extent. The delay of the excretion peak after

consuming broccoli with no of low residual MYR activity has also been ascribed to later dietary GLs hydrolysis that occurred in the intestinal tract (Vermeulen *et al.*, 2006; Vermeulen *et al.*, 2008). Although the metabolism and bioavailability of ITCs have been well studied, little is known about the effect of ITCs concentration, ITCs excretion half-time and maximum excretion peak time on ITCs anticarcinogenic efficiency. It is unclear whether a higher and sudden ITCs release in the body, *e.g.* after consumption of broccoli where MYR is still active, would have a better anticarcinogenic effect than a slower and gradual release *e.g.* after consumption of broccoli with no residual MYR activity. The dose-response curve that correlates the phytochemicals dose and risk of chronic diseases is a U-shaped curve, where the lowest risk of chronic diseases corresponds to an optimal concentration of phytochemical dose. Lower and higher levels than this optimal dose, correspond to an increase of risk of chronic diseases (Holst & Williamson 2008). A limited number of *in vitro* and animal studies indicate a mutagenic potential of broccoli extracts, which might be caused by neoglucobrassicin breakdown products (Latte *et al.*, 2011). Furthermore, *in vitro* and animal studies indicate that certain nitriles might cause damage in specific organs when extremely high concentrations are applied (Latte *et al.*, 2011). However, current studies don't indicate that an increased intake of GLs will lead to toxic effects in humans, especially with focus on increased GR concentrations.

Depending on the desired intake of the ITCs, the air drying conditions can be tuned up to produce broccoli with specific glucoraphanin and MYR profiles. For instance, to produce dried broccoli florets with no MYR activity and high GR concentration (as high as fresh broccoli), the combination of steaming pre-treatment (for 1 min) and drying at low constant conditions such as at 40˚C and 50˚C led to MYR reduction is 85% and 99% (at 40˚C) and 97% and 100% (at 50˚C) and no GR reduction (**Chapter 5**). By increasing the steaming time, a further MYR decrease can be obtained. To produce dried broccoli florets with high MYR activity (as high as fresh broccoli) and high GR concentration (as high as in fresh broccoli), drying at optimized temperature trajectories without any pre-treatment led to 42% and 47% reduction of MYR and no reduction of GR.

Food processing can have positive and negative effects of on food quality but often consumers have a negative perception on industrial food processing. The air drying broccoli case described in this thesis, suggests that food processing does not

necessarily lead to reduction of food nutritional quality but that this might simply result from incorrect optimization strategy.

With the aid of process optimization, the air drying becomes a technology able to stabilize and retain GLs and MYR that may otherwise be lost during processing and even during storage in fresh broccoli.

Implications and future research

Several food processes may need to be optimized to retain the nutritional quality of food. Examples of food processing that may have detrimental effects on food nutritional quality are mentioned in **Chapter 1**. The approach described in this thesis, can be used to improve the nutritional quality as well as the food safety, the shelf life and the sensorial quality. For instance, to optimize the development of flavour compounds during the baking process of bread, by first selecting the desired concentration of flavour compounds, then investigating the kinetics of the relevant formation reactions, followed by the optimizing the baking conditions and eventually testing the optimized baking condition.

The GLs and MYR system is a complicated “healthy system” to preserve, mainly because two compounds are involved, with different heat sensitivity. Despite the simulation results overestimated the GR and MYR degradation and inactivation, the use of a simplified heating system to study the kinetics of their degradation was useful to rule out all the other variables that could interfere, to better understand the fundamental reaction mechanisms. However, it is recommendable to perform validation experiments to test the reaction kinetics in real systems (like in pilot plant with real food).

The similar bioavailability reported after consumption of medium MYR broccoli and high MYR broccoli, shows that medium MYR activity could have similar “ healthy effect” of high MYR product, and that the temperature optimization trajectory of air drying could be modified to the benefit of drying time. The adjective “high”, “medium” and “low” referred to MYR are conventionally used in this thesis to give a measure of the MYR activity. The reader has to take into account that this measure is referred to the residual MYR activity. A fresh broccoli with low MYR activity and

low GLs levels, upon drying, even at the optimized trajectory, may have very low MYR activity.

In fresh *Brassica* vegetables the levels of GLs and MYR can be very different (Charron *et al.*, 2005). The initial GLs concentration and MYR activity are also reduced in fresh vegetables during storage (Song, *et al.*, 2007; Rungapamestry *et al.* 2008). Therefore the opportunity to stabilize both GLs and MYR by using a low-cost drying technology, opens new possibilities for food industry. Such dry product could be used as functional ingredient for dry soups, for seasoning, pasta (Silva *et al.*, 2013), etc.

From ITCs bioavailability perspectives, it would be interesting to investigate the effect of meal components (proteins, fiber, fat, etc.) on ITCs bioavailability. As shown by *in vitro* studies, proteins interact with ITC during digestion depending on the amino acid composition and the ITC type (Kroll *et al*., 1993). The only *in vivo* study available in literature shows that when ITCs are ingested with meat, their adsorption is either 5-fold higher or the same as when ingested without proteins depending on the ITCs (Rungapemestry *et al.*, 2007).

It would be also interesting to compare the extent of the GLs hydrolysis by the gut microflora in vegetarian and omnivorous consumers. Studies report that the microflora of vegetarian subjects has a different composition compared to that of omnivorous subjects (Zimmer *et al.,* 2012). In addition, vegetarian subjects show significantly lower stool pH than omnivorous subjects, which could affect the extent of GLs hydrolysis (Zimmer *et al.,* 2012).

Main conclusions

This thesis describes a case study on air drying of broccoli that starts from the estimation of the kinetic parameters for GLs degradation and MYR inactivation at different water content, passing through air drying optimization to retain these compounds and ending with the investigation of the bioavailability of ITCs after consuming dried broccoli products with different GLs, MYR and ITCs profiles.

This study indicates that a food process that often leads to reduction of nutritional quality (in this thesis, with respect to phytochemicals content), can be optimized,

resulting in products with high nutritional quality. In this case study, GLs were fully retained and MYR and Vc were partially retained upon air drying at optimized temperature trajectories, which resulted in a product where GLs and MYR are much more stable than in fresh broccoli.

Investigations that merge together food technology and human nutrition are scarce. The joint effort of food technology science and human nutrition science, as reported in this study, can be considered a successful example and it is suggested that many other investigations could be performed following the same concept.

References

References

Atuonwu, J. C., Van Straten, G., Van Deventer, H. C., & Van Boxtel, A. J. B. (2011). Optimizing energy efficiency in low temperature drying by zeolite adsorption and process integration. *Chemical Engineering Transactions 25*, 111-116.

Atuonwu J.C., Straten G. van, Deventer H.C. van, Boxtel A.J. B. van. On dryer controllability and energy performance: generalized modelling and experimental validation. Drying Technology 2013. In Press.

Aversa, M., Curcio, S., Calabró, V., & Iorio, G. (2006). An analysis of the transport phenomena occurring during food drying process. *Journal of Food Engineering, 78, (3)*, 922-932.

Bailey, S. D., Bazinet, M. L., Driscoll, J. L., & McCarthy, A. I. (1961). The volatile sulphur components of cabbage. *Journal of Food Science, 26,* 163-170.

Benali, M. (2004). Dehydration of products of biological origin. In A.S. Mujumdar (Ed.), Thermal drying of foods: loss of nutritive content and spoilage issues (pp. 137-152). Enfield, New Hemisphere, USA.

Blandamer, M. J., Engberts, J. B. F. N., Gleeson, P. T., & Reis, J. C. R. (2005). Activity of water in aqueous systems; A frequently neglected property. *Chemical Society Reviews, 34*, 440–458.

Bones, A. M., & Rossiter J. (1996). The myrosinase-glucosinolates system, its organization and biochemistry. *Physiologia Plantarum*, *97*, 194–208.

Bryson, A.E. (1999) Dynamic optimization Editorial Addison Wesley Longman, Menlo Park, CA.

Castenmiller, J. J. M., van de Poll, C. J., West, C. E., Brouwer, I. A., Thomas, C. M.G., van Dusseldorp, M. (2000). Bioavailability of folate from a processed spinach in humans. *Annals of Nutrition and metabolism, 44,* 163-169.

Charron, C. S., Saxton, A. M., & Sams, C. E. (2005). Relationship of climate and genotypeto seasonal variation in the glucosinolate–myrosinase system. II. Myrosinase activity in ten cultivars of *Brassica oleracea* grown in fall and spring seasons. *Journal of the Science of Food and Agriculture, 85,* 682-690.

Cieślik, E., Leszczyńska, T., Filipiak-Florkiewicz, A., Sikora E., & Pisulewski, P. M. (2007). Effects of some technological processes on glucosinolate contents in cruciferous vegetables. *Food chemistry, 105*(3), 976–981.

Clarke, J. D., Hsu, A., Riedl, K., Bella, D., Schwartz, S. J., Steven, J. F., & Ho., E. (2011) Bioavailability and inter-conversion of sulforaphane and erucin in human subjects consuming broccoli sprouts or broccoli supplement in a cross-over study design. *Pharmacological Research, 64,* 456- 46.

Cohen, J. S., & Yang T. C. S. (1995). Progress in food dehydration. *Trends in Food Science & Technology, 6(1),* 20-25.

Combourieu, B., Elfoul, L., Delort, A. M., & Rabot, S. (2001). Identification of new derivatives of sinigrin and glucotropaeolin produced by the human digestive microflora using 1H NMR spectroscopy analysis of *in vitro* incubations. *Drug Metabolism and Disposition, 29,* 1440-1445.

Conaway, C. C., Getahun, S. M., Liebes, L. L., Pusateri, D. J., Topham, D. K. W., Botero-Omary, M., & Chung, F. L. (2000). Disposition of glucosinolates and sulforaphane in humans after ingestion of steamed and fresh broccoli. *Nutrition and Cancer, 38(2),* 168-178.

Cooper, D. A., Eldridge, A. L., & Peters, J. P. 1999. Dietary carotenoids and certain cancers, heart disease, and age-related macular degeneration: A review of recent research. *Nutrition Reviews, 57(7),* 201-214.

Daxenbichler, M. E., Van Etten, C. H., & Spencer, G. F. (1977). Glucosinolates and derived products in cruciferous vegetables. Identification of organic nitriles from cabbage. *Journal of Agricultural and Food Chemistry, 25 (1)*, 121-124.

Dekker, M., Verkerk, R., & Jongen, W. M. F. (2000). Predictive modelling of health aspects in the food production chain: a case study on glucosinolates in cabbage. *Trends in Food Science & Technology 11,* 174–181.

Dekker, M., & Verkerk, R. (2003). Dealing with variability in food production chains: a tool to enhance the sensitivity of epidemiological studies on phytochemicals. *European Journal of Nutrition, 42,* 67-72.

Dekker, M., Hennig, K. & Verkerk R. (2009). Differences in Thermal Stability of Glucosinolates in Five *Brassica* Vegetables. *Czech Journal of Food Sciences, 27, S85-S88.*

Fahey, J. W., Zalcmann, A. T., & Talalay, P. (2001). The chemical diversity and distribution of glucosinolates and isothiocyanates among plants. *Phytochemistry, 56*, 5-51.

Fenwick, G. R., & Heaney, R. K. (1983a). Glucosinolates and their breakdown products in cruciferous crops, foods and feedingstuffs. *Food Chemistry 11*(4), 249–271.

Fenwick, G. R., Griffiths, N. M., & Heaney, R. C. (1983b). Bitterness in Brussels sprouts (*Brassica oleracea* L. var. *gemmifera*): The role of glucosinolates and their breakdown products. *Journal of the Science of Food and Agriculture, 34*(1), 73–80.

Fahey, J. W., Zalcmann, A. T., & Talalay, P. (2001). The chemical diversity and distribution of glucosinolates and isothiocyanates among plants. *Phytochemistry, 56*, 5–51.

Francis, F., Lognay, G., Wathelet, J. P., & Haubruge E. (2002). Characterisation of aphid myrosinase and degradation studies of glucosinolates. *Archives of Insect Biochemistry and Physiology 50,* 173-182.

Ghawi, S. K., Methven, L., Rastall, R. A., Niranjan K. (2011). Thermal and high hydrostatic pressure inactivation of myrosinase from green cabbage: A kinetic study. *Food Chemistry, 131 (4)*, 1240- 1247.

Goula, A. M., & Adamopoulos, K. G. (2013). Retention of ascorbic acid during drying of tomato halves and tomato pulp. *Drying Technology: An International Journal, 24(1),* 57-44.

Hadiyanto, H., Esveld, D. C., Boom, R. M., Van Straten, G., & Van Boxtel, A. G. B. (2008). Product quality driven design of bakery operations using dynamic optimization. *Journal of Food Engineering, 86(3),* 399-413.

Hansson, L. E., Nyren, O., Bergström, R., Wolk, A., Lindgren, A., Baron, J., & 7. Adami, H.O. (1993). Diet and risk of gastric cancer. A population-based case–control study in Sweden. *International Journal of Cancer, 55,* 181–189.

Hernandez, Y., Lobo, M. G., & Gonzalez, M. (2006). Determination of vitamin C in tropical fruits: a comparative evaluation of methods. *Food Chemistry 96(4),* 654-664.

Hendrickx, M., Saraiva, J., Lyssens, J., Oliveira J., & Tobback, P. (1992). The influence of water activity on thermal stability of horseradish peroxidase. *Interational Journal of Food Science and Technology,* 27, 33-40.

Holst, B., & Williamson, G. (2008). Nutrients and phytochemicals: from bioavailability to bioefficacy beyond antioxidants. *Current Opinion in Biotechnology, 19(2),* 73-82.

Hussain, M. M., Dincer, I. (2003). Two-dimensional heat and moisture transfer analysis of a cylindrical moist object subjected to drying: a finite-difference approach. *International Journal of Heat and Mass Transfer, 46(21),* 4033-4039.

International Food Information Council Foundation. (2011) Food & health survey-consumer attitudes toward food safety, nutrition & health.

Jang, M., Hong, E. and Kim, G. H. (2010). Evaluation of antibacterial activity of 3-butenyl, 4-pentenyl, 2-phenylethyl, and benzyl isothiocyanate in *Brassica* vegetables. *Journal of Food Science, 75(7),* 412-416.

Jin, X., Van der Sman, R.G.M. van Straten, G., Boom, R.M., Van Boxtel A.J.B. Optimization of drying trajectories to retain nutritional components in broccoli. Proceedings 18th International Drying Symposium (IDS 2012), Xiamen, China, 11-15 November 2012.

Jin, X., van der Sman, R. G. M., van Maanen, J. F. C., van Deventer, H. C., van Straten, G., Boom, R. M.,& van Boxtel A. J. B. (2013). Moisture sorption isotherms of broccoli interpreted with the Flory-Huggins Free Volume theory. *Food Biophysics (In press*).

Juge, N., Mithen, R. F., Traka, M. (2007). Molecular basis for chemoprevention by sulforaphane: a comprehensive review. *Cellular and Molecular Life Sciences, 64*, 1105-1127.

Karim, O. R. and Adebowale, A.A. (2009). A dynamic method for kinetic model of ascorbic acid degradation during air dehydration of pretreated pineapple slices. *International Food Research Journal*, *16*, 555-560.

Kissen, R., Rossiter, J. T., & Bones, A. M. (2009). The 'mustard oil bomb': not so easy to assemble?! Localization, expression and distribution of the components of the myrosinase enzyme system. *Phytochemistry reviews, 8*, 69–86.

Klibanov, A. M. (1986). Enzymes that work in organic solvents. *Chemical Technology,* June, 354-359.

Kroll, J., Rawel, H., Kröck, R., & Schnaak, W. (1993). Interaction of benzyl isothiocyanate with egg white proteins*. Food / Nahrung, 37(2)*, 179-181.

Kunzek, H., & Vetter, S. (1999). Functional properties of food components and the development of innovative products. *Deutsche Lebensmittel–Rundschau, 97(1)*, 12-22.

Kushad, M. M., Brown, A. F., Kurilich, A. C., Juvik, J. A., Klein, B. P., Wallig, M. A., & Jeffery, E. H. (1999). Variation of glucosinolates in vegetable crops of *Brassica oleracea*. *Journal of Agricultural and Food Chemistry, 47*, 1541–1548.

Latte, K.P., Appel, K.E., & Lampen, A. (2011). Health benefits and possible risks of broccoli - An overview. *Food and Chemical Toxicology, 49(12),* 3287-3309.

Lavelli, V., Zanoni, B., Zaniboni, A. (2007). Effect of water activity on carotenoid degradation in dehydrated carrots. *Food Chemistry, 104,* 1705-1711.

Lewicki, P. P. (1998). Effect of pre-drying treatment, drying and rehydration on plant tissue properties: a review. *International Journal of Food Properties, 1(1)*, 1-22.

Lin, H. J., Probst-Hensch, N. M., Louie A. D., Kau, I. H., Witte, J. S., Ingles, S. A., Frankl, H. D., Lee E. R., & Haile R. W. (1998). Glutathione transferase null genotype, broccoli, and lower prevalence of colourectal adenomas. *Cancer Epidemiol Biomarkers, 7,* 647-652.

Liou, J. K., (1982). An approximate method for nonlinear diffusion applied to enzyme inactivation during drying. PhD thesis, Wageningen University, the Netherlands.

Liu, R.H. (2003). Health benefits of fruit and vegetables are from additive and synergistic combinations of phytochemicals. *American Journal of Clinical Nutrition, 78(3),* 517S-520S.

Ludikhuyze, L., Ooms, V., Weemaes, C., & Hendrickx, W. (1999). Kinetic study of the irreversible thermal and pressure inactivation of myrosinase from broccoli (*Brassica oleracea* L. Cv. *Italica*). *Journal of Agricultural and Food Chemistry, 47,* 1794-1800.

Luning, P. A., Marcelis, W. J., (2009). Food Quality Management: Technological and Managerial Principles and Practices. Wageningen Academic Publishers, Wageningen.

Mari, M., Leoni, O., Bernardi, R., Neri, F., & Palmieri, S. (2008). Control of brown rot on stonefruit by synthetic and glucosinolate-derived isothiocyanates. *Postharvest Biology and Technology, 47 (1)*, 61-67.

Martin, C. (2012). The interface between plant metabolic engineering and human health. *Current Opinion in Biotechnology, 24 (2),* 344-353.

Mennicke, W. H., Gorler. K., Krumbiegel, G., Lorenz, D., & Rittman, N. (1988). Studies on the metabolism and excretion of benzyl isothiocyanate in man. *Xenobiotica, (4),* 441-447.

Mishkin, M., Saguy, I., & Karel M. (1983). Minimizing ascorbic acid loss during air drying with a constraint on enzyme inactivation for a hypothetical foodstuff. *Journal of Food Processing and Preservation, 7,* 193-210.

Mishkin, M., Saguy, I., Karel, M. (1984). Optimization of nutrient retention during processing: Ascorbic acid in potato dehydration. *Journal of Food Science 1984, 49(5),* 1262-1266.

Mithen, R. F., Dekker, M., Verkerk, R., Rabot, S., & Johnson I. T. (2000). The nutritional significance, biosynthesis and bioavailability of glucosinolates in human foods. *Journal of the Science of Food and Agricultural*, *80,* 967-984.

Mithen, R., Faulkner, K., Magrath, R., Rose, P., Williamson, G., & Marquez J. (2003). Development of isothiocyanate-enriched broccoli, and its enhanced ability to induce phase 2 detoxification enzymes in mammalian cells. *Theoretical and Applied Genetics,* 106, (4), 727-734.

Moreno, D. A., Carvajal, M., López-Berenguer, C., & García-Viguera C. (2006). Chemical and biological characterisation of nutraceutical compounds of broccoli. *Journal of Pharmaceutical and Biomedical Analysis, 41(5),* 1508-1522.

Mrkic, V., Radojcic Redovnikovic, I., Mazor Jolic, S., Delonga, K. & Dragovic-Uzelac V. (2010). Effect of drying conditions on indole glucosinolates level in broccoli. *Acta Alimentaria*, *39 (2)*, 167-174.

Munro, I. C., Harwood, M., Hlywka, J. J., Stephen, A., M., Doull, J., Flamm, W G., Adlercreutz, H. (2003). Soy isoflavones: a safety review. *Nutrition reviews, 61(1),* 1-33.

Ndiaye, C., Xu, S.Y., & Wang, Z. 2009. Steam blanching effect on polyphenoloxidase, peroxidase and colour of mango (*Mangifera indica* L.) slices. *Food Chemistry*, *113,* 92-95.

Nelson, K. A., & Labuza, T. P. (1994). Water activity and food polymer science: Implications of state on Arrhenius and WLF models in predicting shelf life. *Journal of Food Engineering, 22(1–4),* 271–289.

Oerlemans, K., Barrett, D. M., Bosch Suades, C., Verkerk, R., & Dekker M. (2006). Thermal degradation of glucosinolates in red cabbage. *Food Chemistry, 95,* 19-29.

Oliver, S., Kuperman, A., & Ozin, G. A. (1998). New model for aluminophosphate formation: transformation of a linear chain aluminophosphate to chain, layer, and framework structures. *Angewandte Chemie International Edition, 37 (1,2),* 46-62.

Oliviero, T., Verkerk, R., & Dekker, M. (2012). Effect of water content and temperature on glucosinolate degradation kinetics in broccoli (Brassica oleracea var. italica). *Food Chemistry*, *132,* 2037-2045.

Oliviero, T., Verkerk, R., Van Boekel, M.A.J.S. & Dekker, M. (2013). Modelling the effect of water content and temperature on inactivation kinetics of myrosinase in broccoli (*Brassica oleracea* var. *italica*), Submitted to *Food Chemistry*.

Pappa, G., Strathmann, J.,Löwinger, M.,Bartsch, H., & Gerhäuser, C. (2007). Quantitative combination effects between sulforaphane and 3,3'-diindolylmethane on proliferation of human colon cancer cells *in vitro*. *Carcinogenesis, 28(7),* 1471-1477.

Pellegrini, N., Chiavaro, E., Gardana, C., Mazzeo, T., Contino, D., Gallo, M., Riso, P., Fogliano, V., & Porrini, M. (2010). Effect of Different Cooking Methods on Colour, Phytochemical Concentration, and Antioxidant Capacity of Raw and Frozen *Brassica* Vegetables. *Journal of Agricultural and Food Chemistry*, *58,* 4310-4321.

Perera, C.O. (2005). Selected Quality Attributes of Dried Foods. *Drying Technology: An International Journal, 23,* 717-730.

Raso, J., Heinz, V. (Eds.), (2007). Pulsed Electric Fields Technology for the Food Industry – Fundamentals and Applications, Food Engineering Series, Springer, Heidelberg.

Ragaert, P., Verbeke, W., Devlieghere, F., & Debevere, J. (2004). Consumer perception and choice of minimally processed vegetables and packaged fruits. *Food Quality and Preference, 15(3),* 259-270.

Rossi, M. , Giussani, E., Morelli, R., Lo Scalzo, R., Nani, R.C., & Torreggiani, D. (2003). Effect of fruit blanching on phenolics and radical scavenging activity of highbush blueberry juice. *Food Research International, 36(9-10),* 999-1005.

Rouzaud, G., Young, S. A., & Duncan A. J. (2004). Hydrolysis of glucosinolates to isothiocyanates after ingestion of raw or microwaved cabbage by human volunteers. *Cancer Epidemiology, Biomarkers & Prevention, 13,* 125-131.

Rungapamestry, V., Duncan, A. J., Fuller, Z., & Ratcliffe, B. (2006). Changes in glucosinolate concentrations, myrosinase activity, and production of metabolites of glucosinolates in cabbage (*Brassica oleracea* var. *capitata*) cooked for different durations. *Journal of Agricultural and Food Chemistry, 54 (20),* 7628-7634.

Rungapamestry, V., Duncan, AJ., Fuller, Z., & Ratcliffe B. 2007. Effect of meal composition and cooking duration on the fate of sulforaphane following consumption of broccoli by healthy human subjects. *British Journal of Nutrition, 97,* 644-652.

Rungapamestry, V., Duncan A. J., Fuller, Z., & Ratcliffe, B. (2008). Influence of blanching and freezing broccoli (*Brassica oleracea* var. *italica*) prior to storage and cooking on glucosinolates concentrations and myrosinase activity. *European Food Research and Technology, 227*, 37-44.

Sablani, S. S. (2006). Drying Technology. Drying of fruits and vegetable: retention of nutritional/functional quality. *Drying Technology: An International Journal, 24*, 123-125.

Shapiro, T. A., Fahey, J. W., Wade, K. L., Stephenson, K. K., & Talalay P. (1998). Human metabolism and excretion of cancer chemoprotective glucosinolates and isothiocyanates of cruciferous vegetables. *Cancer Epidemiology, Biomarkers & Prevention, 7*, 1091-1100.

Shapiro, T. A., Fahey, J. W., Wade, K. L., Stephenson, K. K., & Talalay, P. (2001). Chemoprotective Glucosinolates and Isothiocyanates of Broccoli Sprouts Metabolism and Excretion in Humans. *Cancer Epidemiology, Biomarkers & Prevention, (10),* 501–508.

Shahidi, F., Naczk, M., Myhara, R. M. (1990). Effect of processing on the soluble sugars of brassica seeds. *Journal of Food Science, 55 (5),* 1470-1471.

Schmalko, M. E., Scipioni, P. G., & Ferreyra D. J. (2007). Effect of water activity and temperature in colour and chlorophylls changes in yerba mate leaves. *International Journal of Food Properties, 8(2),* 313-322.

Silva, E., Sagis, L. M. C., Van der Linden, E., Scholten, E. (2013). Effect of matrix and particle type on rheological, textural and structural properties of broccoli pasta and noodles. *Journal of Food Engineering, 119(1)*, 94-103.

Song, L., & Thornalley, P. J. (2007). Effect of storage, processing and cooking on glucosinolate content of *Brassica* vegetables. *Food and Chemical Toxicology, 45,* 216-224.

Steinmetz, K. A., Potter, J. D. (1996) Vegetables, fruit and cancer prevention: a review. *Journal of the American Dietetic Association 96,* 1027-1039.

Sunjka, P., Raghavan, G. S. V., & Orsat, V. (2008). Drying pretreatments: a review of methods and their influence on the process. 16th International Drying Symposium. Hyderabad, India 9-12 November 2008.

Tian, Q., Rosselot, R. A., & Schwartz, S. J. (2005). Quantitative determination of intact glucosinolates in broccoli, broccoli sprouts, Brussels sprouts, and cauliflower by high-performance liquid chromatography–electrospray ionization–tandem mass spectrometry. *Analytical Biochemistry, 343,* 93-99.

Traka, M., & Mithen, R. (2009). Glucosinolates, isothiocyanates and human health. *Phytochemisty Reviews, 8(1),* 269-282.

Turkmen, N., Sari, F., Velioglu. Y. S. (2005). The effect of cooking methods on total phenolics and antioxidant activity of selected green vegetables. *Analytical, Nutritional and Clinical Method, 93(4)*,713-718.

Uddin, M. S., Hawlader, M. N. A., Luo Ding, & Mujumdar, A. S. (2002). Degradation of ascorbic acid in dried guava during storage. *Journal of Food Engineering, 51(1),* 21-26.

Vallejo, F., Tomás-Barberán, F. A., & García-Viguera, C. (2002). Glucosinolates and vitamin C content in edible parts of broccoli florets after domestic cooking. *European Food Research and Technology, 215,* 310-316.

Van Boekel, M. A. J. S. (2009). *Kinetic modelling of reactions in food.* Taylor & Francis Group, Boca Raton, FL.

Van Boekel M. A. J. S. , Fogliano, V., Pellegrini, N., Stanton, C., Scholz, G., Lalljie S., Somoza, V., Knorr, D., Jasti, P. R., & Eisenbrand G. (2010). A review on the beneficial aspects of food processing. *Molecular Nutrition and Food Research, 54,* 1215-1247.

Van Boxtel, A.J.B., Otten, Z.E.H., & van der Linden, H.J.L.J. (1992). Dynamic optimization of a one-stage reverse osmosis installation with respect to membrane fouling. *Journal of Membrane Science, 65,* 277-293

Van Eylen, Oey, I., Hendrickx, M., & Van Loey, A. (2006). Temperature and pressure stability of mustard seed (*Sinapis alba* L.) myrosinase. *Food Chemistry, 97,* 263-271.

Van Eylen, D. E., Oey, I., Hendrickx, M., & Van Loey, A. (2007). Kinetics of the stability of broccoli (*Brassica oleracea* cv. *italica*) myrosinase and isothiocyanates in broccoli juice during pressure/temperature treatments. *Journal of Agricultural and Food Chemistry 55,* 2163-2170.

Van Eylen, D., Oey, I., Hendrickx, M.,& Van Loey, A (2008). Effects of pressure/temperature treatments on stability and activity of endogenous broccoli (*Brassica oleracea* L. cv. *Italica*) myrosinase and on cell permeability. *Journal of Food Engineering, 89,* 178-186.

Van het Hof, K. H., Gartner, C., West, C. E., Tijburg, L. B. (1998). Potential of vegetable processing to increase the delivery of carotenoids to man. *International Journal for Vitamin and Nutrition Research. 68,* 366-370.

Vega-Gálvez, A., Di Scalab, K., Rodrígueza, K., Lemus-Mondacaa, R., Miranda, M., López, J., Perez-Wona M. (2009). Effect of air-drying temperature on physico-chemical properties, antioxidant capacity, colour and total phenolic content of red pepper (*Capsicum annuum,* L. var. *Hungarian*). *Food Chemistry, 117*(*4*), 647-653.

Verhoeven, D. T. H., Verhagen, H., Goldbohm, R. A., van Brandt, P. A., & van Poppel, G. (1997). A reviewof mechanisms underlying anticarcinogenicity by *Brassica* vegetables, *Chemico-Biological Interactions. 103,* 79-129.

Verkerk, R., Dekker, M., & Jongen, W. M. F. (2001). Post-harvest increase of indolyl glucosinolates in response to chopping and storage of *Brassica* vegetables. *Journal of the science of Food and Agriculture, 81,* 953-958.

Verkerk, R. (2002). Evaluation of glucosinolate levels throughout the production chain of *Brassica* vegetables; Towards a novel predictive modelling approach. PhD dissertation, Wageningen University, Wageningen, The Netherlands.

Verkerk, R., & Dekker, M. (2004). Glucosinolates and Myrosinase Activity in Red Cabbage (*Brassica oleracea* L. Var. *Capitata* f. *rubra* DC.) after Various Microwave Treatments. *Journal of Agricultural and Food Chemistry, 52*, 7318-7323.

Verkerk, R., Schreiner, M., Krumbein, A., Ciska, E., Holst, B., Rowland, I., De Schrijver, R., Hansen, M., Gerhäuser, C., Mithen R., & Dekker. M. (2009). Glucosinolates in *Brassica* vegetables: The influence of the food supply chain on intake, bioavailability and human health. *Molecular Nutrition & Food Research, 53,* S219 -S265.

Verkerk, R., Knol, J. J., & Dekker, M. (2010). The effect of steaming on the glucosinolate content in broccoli. *Acta Horticulturae, 867,* 37-46.

Vermeulen, M., van den Berg, R., Freidig, A. P., van Bladeren, P. J. & Vaes, W. H. J. (2006). Association between consumption of cruciferous vegetables and condiments, and excretion in urine of isothiocyanate mercapturic acids. *Journal of Agricultural and Food Chemistry, (54),* 5350-5358.

Vermeulen, M., Klöpping-Ketelaars, I. W. A. A., van den Berg, R., & Vaes, W. H. J. (2008). Bioavailability and kinetics of sulforaphane in humans after consumption of cooked versus raw broccoli. *Journal of Agricultural and Food Chemistry, 56 (22),* 10505-10509.

Vermeulen, M., Boerboom, A. M. J. F., Blankvoort, B. M. G., Aarts, J. M. M. J. G., Rietjens, I. M. C. M., Van Bladeren, P. J., & Vaes, W. H. J. (2009). Potency of isothiocyanates to induce luciferase reporter gene expression via the electrophile-responsive element from murine glutathione S-transferase Ya. *Toxicology in Vitro, 23 (4)*, 617-621.

Villota, R. & Karel, M. (1980). Prediction of ascorbic acid retention in a model system. 11. Simulation of retention in a model system. *Journal of Food Processing and Preservation, 4*, 141.

Wallenberg, L. W. (1992). Inhibition of carcinogenesis by minor dietary constituents. *Cancer Research (Suppl.), 52,* 2085s-2091s.

Wandel, M. (2007). Consumer concern and behaviour regarding food and health in Norway. *International Journal of Consumer Studies, 18 (3)*, 203-215.

Wang, J. M., Rosen, R. T. & Ho, C. T. (1999). Thermal degradation of sulforaphane in aqueous solution. *Journal of Agricultural and Food Chemistry, 47 (8)*, 3121-3123.

Wang, L. S., & Stoner, G. D. (2008). Anthocyanins and their role in cancer prevention. *Cancer Letters, 269(2),* 281–290.

Wechtersbach, L., & Cigic, B. (2007). Reduction of dehydroascorbic acid at low pH. *Journal of Biochemical and Biophysical Methods, 70,* 767-772.

West, L., Tsui, I., & Haas G. (2002). Single column approach for the liquid chromatographic separation of polar and non-polar glucosinolates from broccoli sprouts and seeds. *Journal of Chromatography A, 966(1–2),* 227–232.

Zanoni, B., Peri, C., Nani, R., & Lavelli, V., (1998). Oxidative heat damage of tomato halves as affected by drying. *Food Research International, 31(5),* 395-401.

Zhang, Y., & Talalay, P. (1994). Anticarcinogenic activities of organic isothiocyanates: chemistry and mechanisms. *Cancer Research (Suppl.), 54*, 1976s–1981s.

Zimmer, J., Lange, B., Frick, J-S., Sauer, H., Zimmermann, K., Schwiertz, A., Rusch., Klosterhalfen, K. S., & Enck, P. (2012). A vegan or vegetarian diet substantially alters the human colonic faecal microbiota. *European Journal of Clinical Nutrition, 66,* 53-60.

Zimmerman, S. B. and Minton, A. P. (1993). Macromolecular crowding and confinement: biochemical, biophysical, and potential physiological consequences. *Annual Review of Biophysics and Biomolecular Structure, 22*, 27-75.

Summary

Background

The main purpose of industrial food processing is to produce safe and high quality food as demanded by consumers, mainly by inactivating microorganisms, toxins, allergens and enzymes while improving sensorial properties. However food processing can lead to the formation of undesired compounds and loss of freshness and related healthy and sensory attributes. The concentration of healthy compounds present in *Brassica* vegetables can be reduced by industrial and domestic processing. *Brassica* vegetables, *e.g.* broccoli, cabbage, cauliflower, are rich in glucosinolates (GLs), secondary metabolites that can be hydrolysed by a group of endogenous β-glucosidases termed myrosinase (MYR) upon damage of the plant matrix (mastication, mincing etc.). Among the breakdown products, isothiocyanates (ITCs) are considered healthy compounds. *In vitro*, *in vivo* and epidemiological studies show the protective effect of isothiocyanates against several types of cancer. Air drying is a common method to extend shelf life with the possibility of storing dry food at room temperature, allowing consumption of vegetables out of season. Air drying can affect the health- promoting effect by reducing GLs content, MYR activity and ITCs formation due to the use of high temperatures combined with a high air flow velocity.

Aim

The first aim of this study was to kinetically model the effect of temperature and water on GLs degradation and MYR inactivation in broccoli (*Brassica oleracea* var. *italica*). These models were used to optimize air drying conditions to obtain broccoli product with certain GLs levels and MYR activity. The second aim was to investigate the absorption of ITCs after consuming broccoli products with different GLs, MYR and ITCs profiles. In this way, health effects were assessed *in vivo.*

Results

During air drying, broccoli is subject to changes in water content and temperature that affect the occurrence of GLs and MYR.In order to be able to optimize drying process conditions to produce dried broccoli with specific GLs and MYR profiles, thermal degradation and inactivation kinetics of GLs and MYR in broccoli with different water content were studied. (**Chapter 3 and 4**). Batches of broccoli with different water content were incubated for various times at different temperatures and GLs concentration was monitored (**Chapter 3**). GLs degradation could be described by

first-order kinetics. GLs degradation rate constants increased with temperature for all the water contents investigated. Water content was found to have a remarkable effect on GLs kinetic parameters. The lowest degradation rate constants were found in the driest broccoli (13% water content) at temperatures ≤ 100°C and an increase in degradation rate constants was observed as the water content decreased (from 34 up to 82% water content). This effect of water on reaction rate constants may be explained by the change of the activity coefficient of reagents. The rate of a reaction in non-ideal solution, such as broccoli, depends on the activity coefficients of reactants and that of the activated complex. The activity coefficients of GLs increase as the a_w decreases because of the volume exclusion effect on GLs . If the increase in the activity coefficient of GLs is high enough, as probably in this case, the rate of the hydrolysis reaction increases despite the decrease of the activity coefficient of water, explaining the order of degradation rates of GLs observed at temperatures ≤ 100°C. At 120°C the inverse was observed with the driest broccoli showing the highest GLs degradation rate constants. In the driest broccoli the GLs hydrolysis reaction could be diffusion-limited due to the very low a_w. In such case, at higher temperatures, as the molecular mobility increases, the reaction rate would be less diffusion-limited, contributing to an increase of the degradation rate constants of the driest broccoli at 120°C.

MYR inactivation kinetics were studied on batches of broccoli with different water content, incubated for various times at different temperatures (**Chapter 4**). MYR inactivation could be described by a two consecutive steps reaction model. This model describes an enzyme inactivation that consist of two irreversible reactions. In the first step the native enzyme structure changes to an intermediate form with lower activity than the native form and in the second step, the intermediate form irreversibly changes to the inactive form. MYR inactivation rate constants also increased with temperature for all water content investigated. Also for MYR, the water content had a large effect on the inactivation rate constants. MYR inactivation rate constants were lower in the driest samples (10% water content) at all the studied temperatures. Broccoli with 31%, 67% and 90% water content showed similar inactivation rate constants. The MYR stability in the driest broccoli may be explained by low conformational mobility of MYR in a low water content broccoli. In a molecular crowded system, as a low water content system, there may be no sufficient volume for an enzyme to unfold and loss its activity.

Then, the kinetic parameters of glucoraphanin (GR) degradation (the most bioactive GLs, ref) and MYR inactivation estimated in the previous studies, along with kinetic parameters for Vitamin C degradation taken from literature, were used to optimise air drying conditions (**Chapter 5**). Dynamic optimization was applied to find the temperature trajectories that minimize GR and Vitamin C degradation and MYR inactivation. The obtained optimized temperature trajectories were also experimentally tested to monitor GR and MYR activity. Simulation and experimental results for optimized temperature trajectories were compared to constant inlet air drying temperature at 40 and 50°C. The optimized temperature trajectories led to remarkable improvement of the retention. However, the mathematical simulation of drying at both the optimized temperature trajectories and at constant temperature tended to overestimate the GR degradation and MYR inactivation. The overestimation was probably due to the different heating treatments. The simulation was obtained by data of kinetics measured by heating powdered broccoli in a simplified system such as metal tubes in heating block. Whereas to test the optimization trajectory and constant temperature drying, intact broccoli florets were dried in a pilot air dryer. The plant matrix of intact florets may have affected the GLs degradation and MYR inactivation during drying. In intact florets GLs are localised in the vacuoles and MYR is localized in special vacuole-like myrosin cells, whereas upon grinding this structure is lost. The release of the compounds may have enhanced their degradation or inactivation, whereas the plant structure may have had a protective effect on GR and MYR. The different heat treatments may also have affected GLs degradation and MYR inactivation. During drying water evaporates from the surface of broccoli and from the inside to the outside of broccoli. As a result, the concentration of reactants increase and at the same time a concentration gradient arises. In addition, in intact broccoli also transport due to the capillary action may occur. These changes in concentration will influence the reaction kinetics. The simplified heating system can be considered a static system, the heating tubes were closed, so all the transport phenomena and the changes in concentration were reduced.

To investigate *in vivo* the effect of broccoli products with different GLs, MYR and ITC profiles, the actual ITC absorption (excretion kinetics and bioavailability), resulting from the consumption of these broccoli products, was systematically and quantitatively studied (**Chapter 6**). Broccoli batches were differently processed in order to obtain five dried products with different GLs, MYR and ITC profiles. These products were consumed by 15 human volunteers in a cross-over design study. GR,

glucoiberin (GB) (one of the GLs present in broccoli) and their ITCs, sulforaphane (SR) and iberin (IB), were determined in the broccoli products and their excreted metabolites in urine (ITCs conjugates). In particular, the role of different MYR activity in the broccoli products on ITC formation, bioavailability and excretion kinetics was investigated. The first broccoli product had high concentrations of pre-formed SR and IB. In the other four broccoli products the GR and GI concentration was similar, whereas MYR activity was high (100%), medium (20%), low (2%) and inactivated. The consumption of broccoli product with 20% MYR activity showed similar bioavailability as the broccoli product with intact MYR activity. Moreover, the consumption of low MYR broccoli product showed significantly higher bioavailability values than the broccoli product with no residual MYR activity. These results suggest that even low residual MYR activities in the broccoli product are sufficient to hydrolyse GR and GI during mastication, highly contributing to the formation of SR and IB, and therefore to their final bioavailability. In addition, the excretion peaks of SR and IB conjugates after ingestion of broccoli with no or low residual MYR activity, appeared 1-3 h later than after ingestion of broccoli products with intact MYR activity. The excretion of SR and IB conjugates and the delay of their excretion peaks in urines after consumption of no residual MYR activity product, confirmed that GLs can be hydrolysed in the human gut by the MYR-like activity of human enteric microflora, even though to a much lower extent.

Conclusions

This study shows that air drying broccoli can be tuned up depending on the desired bioavailability of ITCs resulting from dry broccoli consumption. However, this study also shows that this approach can be applied to other food processing. The concentrations of desired compounds in the final product can be monitored and predicted by selecting the desired compounds of a food, by estimating the kinetic parameters of their degradation and applying dynamic optimization. Eventually, in case of healthy compounds, their bioavailability could be tested to study *in vivo* the effect of certain concentrations. The joint effort of food technology science and human nutrition science, reported in this study, can be considered a successful example and it is suggested that many other investigations could be performed following the same concept.

Samenvatting

Achtergrond

Een belangrijke reden om op industriële wijze levensmiddelen te produceren is om voedsel van hoge kwaliteit en veiligheid te garanderen, zoals vereist voor consumenten, voornamelijk door inactiveren van micro-organismen, giftige stoffen, allergenen en enzymen. Echter, de verwerking van levensmiddelen kan leiden tot de vorming van ongewenste stoffen en het verlies van versheid en daaraan verwante gezondheids- en sensorische aspecten. De concentratie van gezonde bestandsdelen die in *Brassica* groenten aanwezig zijn kan worden verlaagd door industriële en huishoudelijke behandelingen. Groenten van de *Brassica* familie zijn rijk aan glucosinolaten (GLs), secundaire metabolieten, die gehydrolyseerd kunnen worden door endogene β-glucosidases, myrosinases (MYR) genaamd. Deze hydrolyse vindt plaats wanneer de matrix van de plant beschadigd wordt, bijvoorbeeld door kauwen. Van de bestandsdelen die gevormd worden bij deze hydrolyse, worden isothiocyanaten (ITCs) gezien als gezondheidsbevorderende stoffen. Zowel *in vitro, in vivo* en epidemiologische studies hebben aangetoond dat ITCs een beschermend effect hebben tegen verschillende soorten kanker.

Drogen is een veelgebruikte methode bij de productie van veel voedingsmiddelen om de houdbaarheid bij kamertemperatuur te verlengen, waarmee consumptie buiten het seizoen mogelijk wordt. Het drogen van *Brassica* groenten kan de gezondheidsbevorderende effecten beïnvloeden door de hoeveelheid GLs, de MYR activiteit en de vorming van ITCs te verminderen als gevolg van de hoge temperaturen en de hoge luchtstroom.

Doel

Het eerste doel van dit onderzoek was het ontwerpen van een kinetisch model voor de effecten van temperatuur en water op de enzymatische omzetting van GLs naar ITCs en de inactivatie van het hydrolytische enzym myrosinase in broccoli (*Brassica oleracea* var. *Italica*). Dit mathematisch model wordt gebruikt om de omstandigheden tijdens drogen te optimaliseren, zodat er broccoli met constante hoeveelheid GLs en MYR activiteit kan worden verkregen. Het tweede doel van deze studie was om de absorptie van ITCs te onderzoeken na consumptie van broccoli met verschillende GLs, MYR en ITCs profielen. Op deze manier konden gezondheidseffecten *in vivo* worden onderzocht.

Resultaten

Tijdens het drogen ondergaat broccoli veranderingen in de hoeveelheid water en de temperatuur hetgeen de hoeveelheid GLs en de activiteit van MYR beïnvloedt. Om het droogproces te kunnen optimaliseren, en daardoor broccoli met bepaalde concentraties GLs en MYR activiteit te kunnen produceren, zijn de mate van activiteit van MYR en afbraak van GLs door middel van warmte bestudeerd (**Hoofdstuk 3 en 4).** Broccoli batches met verschillende hoeveelheden water zijn verhit bij verschillende temperaturen en tijden (**Hoofdstuk 3**). De afbraak van de GLs tijdens verhitting kan worden beschreven als een eerste orde reactie. Bij alle broccoli batches nam de snelheid voor afbraak van glucosinolaten toe bij toenemende temperaturen. De laagste afbraak is gevonden in broccoli met het laagste watergehalte (13% water) bij temperaturen onder de 100°C; de afbraaksnelheid nam toe bij een toenemend watergehalte in de broccoli (34% tot 82% water). Dit effect van water op de mate van afbraak van glucosinolaten kan worden verklaard door de verandering van de activiteitscoëfficiënten van de reactie componenten. De mate van reactie in een niet homogene oplossing, zoals broccoli, hangt af van de activiteitscoëfficiënten van de reagentia en het geactiveerde complex. De activiteitscoëfficiënt van GLs neemt toe wanneer de wateractiviteit afneemt. Als deze toename groot genoeg is, zoals waarschijnlijk in dit geval, dan neemt de mate van de hydrolyse reactive toe ondanks de afname van de activiteitscoëfficiënt van water. Dit verklaart de mate van afbraak van GLs, gevonden bij temperaturen onder de 100°C. Bij 120°C werd juist het tegenovergestelde geobserveerd, dus de broccoli met de laagste wateractiviteit die de hoogste mate van afbraak had. In de droogste broccoli zou de hydrolyse van de GLs gelimiteerd zijn door diffusie, door de lage wateractiviteit. In zulke gevallen, bij hogere temperaturen zorgt de moleculaire mobiliteit ervoor de reactie minder gelimiteerd wordt door diffusie.

De kinetiek van MYR inactivatie is onderzocht in batches broccoli met verschillende wateractiviteit, die zijn verhit gedurende verschillende tijden op bepaalde temperaturen (**Hoofdstuk 4**). De afname in MYR activiteit kan worden beschreven door een model met twee opeenvolgende stappen. Dit model beschrijft een enzym inactivatie die bestaat uit twee onomkeerbare reacties. In de eerste stap verandert het oorspronkelijke enzym naar een tussenvorm met een lagere activiteit en in de tweede stap wordt de tussenvorm omgezet naar een inactief enzym. De mate

van inactivatie nam toe bij een toenemende temperatuur bij alle onderzochte wateractiviteiten van de broccoli. Ook voor het MYR enzym had de hoeveelheid water een groot effect op de mate van inactivatie. De reactiesnelheidsconstanten was lager in de droogste monsters (10% water) bij alle onderzochte temperaturen. Voor broccoli met 31%, 67% en 90% water waren de snelheidsconstanten ongeveer gelijk. In een reactie systeem met weinig water, is er waarschijnlijk niet genoeg ruimte voor een enzym om zich te ontvouwen en activiteit te verliezen.

De kinetische parameters voor afbraak van glucoraphanin (GR, de meest bioactieve GL) en MYR inactivatie, samen met kinetische parameters voor afbraak van vitamine C uit de literatuur, zijn gebruikt om de condities voor drogen te optimaliseren (**Hoofdstuk 5**). Dynamische optimalisatie was toegepast om temperatuur trajecten te identificeren die de afbraak van GR en vitamine C en inactivatie van MYR minimaliseren. De verkregen temperatuur trajecten zijn experimenteel getest voor GR gehalte en MYR activiteit.

Resultaten uit simulatie studies en experimenten van geoptimaliseerde temperatuurtrajecten zijn vergeleken met drogen bij een constante temperatuur van 40 en 50°C. De geoptimaliseerde temperatuurtrajecten leidden tot een opmerkelijke verbetering van behoud van GR in gedroogde broccoli. Echter, de simulatie studies leken in de meeste gevallen een overschatting te geven van de GR afbraak en MYR inactivatie. Deze overschatting was waarschijnlijk te wijten aan verschillende manieren van verhitten. De simulatie was uitgevoerd met kinetische data verkregen door verhitten van broccolipoeder in een vereenvoudigd verhittingssysteem met metalen buizen. Terwijl het experiment met geoptimaliseerde temperatuurtrajecten uitgevoerd is intacte broccoli gedroogd in een semi-industriële droger. De matrix van de plant kan de afbraak van GLs en inactivatie van MYR hebben beïnvloed. In intact broccoli weefsel zijn de GLs gelokaliseerd in de vacuolen en het enzym MYR is aanwezig in speciale vacuole-achtige myrosin cellen waarvan de structuur na vermalen kapot gaat**.** Het vrijkomen van deze stoffen heeft mogelijk hun afbraak of inactivatie bevorderd, terwijl de plant structuur een beschermende werking heeft voor GR en MYR. Verschillende hittebehandelingen kunnen ook een effect hebben op de afbraak van GLs en MYR inactivatie. Tijdens het drogen verdampt water vanaf het oppervlak van de broccoli en vanaf de binnenkant naar de buitenkant van de broccoli. Hierdoor neemt de concentratie van reactanten toe en op het zelfde

moment ontstaat er een concentratiegradiënt. Daarnaast speelt ook de capillaire werking een rol in het transport in de broccoli. Deze concentratieveranderingen hebben invloed op de reactiesnelheden. Het vereenvoudigde verhittingssysteem kan gezien worden als een statisch systeem; de verhittingsbuizen waren gesloten waardoor het totale transport en concentratieverandering werd verminderd. Om *in vivo het* gezondheidseffect van broccoli te onderzoeken, zijn producten met verschillende GLs, MYR en ITC hoeveelheden gebruikt waarvan de ITC absorptie (excretie en biologische beschikbaarheid) als gevolg van broccoli consumptie systematisch en kwantitatief werd bestudeerd (**Hoofdstuk 6**). Broccoli batches zijn verwerkt teneinde 5 gedroogde broccoli producten te verkrijgen met verschillende GLs, MYR en ITC hoeveelheden. Deze producten zijn door 15 vrijwilligers geconsumeerd in een cross-over studie. De glucosinolaten glucoraphanin (GR) en glucoiberin (GI) (de belangrijkste GLs aanwezig in broccoli) en hun isothiocyanaten, sulforophane (SR) en iberin (IB) zijn geanalyseerd in de gedroogde broccoli producten en in de uitgescheden metabolieten in urine (ITCs conjugaten). Met name is de rol van verschillende MYR activiteiten op de vorming van ITCs, biologische beschikbaarheid en uitscheiding van metabolieten in urine onderzocht. Het eerste broccoli product had een hoge concentratie aan SR en IB. In de andere vier broccoli producten waren de GR en GI concentraties vergelijkbaar, terwijl de MYR activiteit hoog (100%), medium (20%), laag (2%) en geïnactiveerd was. Consumptie van het product met 20% MYR activiteit had vergelijkbare biologische beschikbaarheid in vergelijking met de broccoli waar de MYR activiteit intact was. Bovendien hadden de producten met een lage MYR een significant hogere biologische beschikbaarheid dan de broccoli zonder overgebleven MYR activiteit. Deze resultaten suggereren dat zelfs lage MYR activiteiten in de gedroogde broccoli producten genoeg zijn om de glucosinolaten GR en GI the hydrolyseren tijdens kauwen en hiermee veel bijdragen aan de vorming van de isothiocyanaten SR en IB en vervolgens aan hun uiteindelijke biologische beschikbaarheid. Tevens is de uitscheiding van SR en IB conjugaten na inname van broccoli zonder MYR activiteit of met een lage MYR activiteit ongeveer 1 tot 3 uur later dan de conjugaten afkomstig van broccoli producten met MYR activiteit. Deze vertraagde uitscheiding van isothiocyanaat conjugaten duidt erop dat GLs in beperkte mate gehydrolyseerd kunnen worden in de menselijke darm door de MYR-achtige activiteit van inwendige microflora.

Conclusie

Dit onderzoek laat zien dat het drogen van broccoli kan worden geoptimaliseerd naar de gewenste biologische beschikbaarheid van bioactieve isothiocyanaten. Deze studie laat zien dat deze benadering ook toegepast kan worden op andere vormen van voedsel verwerking. De concentratie van de gewenste componenten in het uiteindelijke product kan worden beheerst en voorspeld door de kinetische parameters voor afbraak van de gewenste componenten te schatten en een dynamische optimalisatie toe te passen.

Uiteindelijk kan de biologische beschikbaarheid van bioactieve componenten kan onderzocht om de *in vivo* effecten van verschillende concentraties te bepalen. De samenwerking tussen technologische- en humane voedingswetenschappen in dit onderzoek kan worden beschouwd als een succesvol voorbeeld en toepasbaar ik soortgelijke concepten.

Acknowledgements

Everything started the 5th of May 2009. It has been a long and busy trip that has definitely changed my life. Moving to another country, meeting people from all over the world, learning new things and tackling the responsibility of conducting and finalizing a PhD project. I'm proud of me. I'm even more proud of me thinking that when I started I didn't know whether or not I was able to finalize my PhD.

Nonetheless the accomplishment of a PhD, is not only due to the efforts of the candidate but also to all the people who, directly and indirectly involved, support her/him.

It hasn't been an easy trip, but it would have been worse without my supervisors.

Matthijs Dekker and **Ruud** Verkerk, thank you for being always helpful and supportive. Your positive attitude has been crucial when I had to tackle things that looked impossible to accomplish to me. Scientifically or humanly you have been always next to me. I will never forget it.

Tiny van Boekel, during these years, we did not have many meetings. Nevertheless, you played an important role. Your book on kinetic modelling has been a fundamental tool to accomplish my PhD. Moreover, you made the last weeks before handing in the thesis reading version, much easier than I expected. You were there in time and carefully commented the introduction and discussion chapters. Thanks!

Ton van Boxtel**, Xin** Jin and **James** Atuonwu you were part of the project EOSLTe7043. It was a real pleasure to collaborate with you. Kind, efficient and loyal, that is what I think about you. **Xin**, although you move back to China to work at Soochow University, where I'm sure you will have a successful academic career, I wish we will keep in contact.

Martijn Vermeulen, thanks a lot for your help. You have played an important role to accomplish the human intervention study.

A special thank goes to the technicians of FQD: **Charlotte** van Twisk, **Xandra** Bakker, **Frans** Lettink and **Geert** Meijer thank you for facilitating my work during these years. **Xandra** and **Frans**, you helped me during the hardest moment of my PhD with the last experiments, thank you a lot!

Milo Vermetten and **Ralf** Meij, thank you for translating my Summary in Dutch! I hope you will keep nice memories of the time we spend together as I will.

I should also thank all the students I have been supervising during these years and that gave a contribution to my thesis: **Agostino, Diogo, Weluzani, Gert, Javi, Xun**.

To **all my PhD colleagues and the other employees from FQD and FPH**, it was very nice to share this experience with you.

A special thank goes to my glucosinolate colleagues **Kristin** Hennig, **Irmela** Kruse and **Radhika** Bongoni. You have been for me much more than loyal colleagues. We shared so much, and we always have been helping each other. I will never forgot all the times you cheered me up.

Gilda, **Stefano**, **Giulia**, **Van Ahn**, **Yul**, **Dieuwerke**, **Vincenzo**, **Iliana**, **Irma**, in a way or another you supported me and cheered me up. **Iliana**, I have really appreciated your help in editing my thesis and designing the cover. Thank you all!

Edoardo, together we decided to move to Wageningen, together we faced all the difficult and the happy moments. You have been always willing to support me, emotionally and scientifically. Your support has been crucial for my PhD, you are the one I should thank the most.

Un grazie speciale va anche alla mia famiglia (relatives). Ai miei genitori **Vincenzo** e **Anna Maria**, a mia sorella **Simona**, **Rachid** ed il mio meraviglioso nipotino **Amir**. Anche alle mie amiche che mi sono sempre state vicine: **Claudia**, **Elisa**, **Maria**, **Maria Teresa** e **Loredana**. “Cosi lontani, cosí vicini”.

About the author

Teresa Oliviero was born on 2nd of February 1982 in Torre Del Greco (Naples), Italy. She grew up in Ercolano (Naples) and she finished high school in 2001. She started the Bachelor's program in 'Food Technology' at Federico II University of Naples. After the Bachelor graduation, she enrolled in the Master program 'Food Sciences and Technologies'. During the Master program she won a grant to participate to the Erasmus program. During the Erasmus, Teresa worked on part of her Master thesis at Institute for Food Technology and Food Chemistry Technical University Berlin (TUB). The Master thesis of Teresa was on "Antioxidant activity and Maillard reaction products during roasting of cocoa beans model systems". Afterwards, she returned in Naples to finalize the Master program and in 2007 she received the Master degree. After the graduation, Teresa was granted a short term scientific mission to Ankara for an internship at Food Engineering department, Hacettepe University, Beytepe, where she worked on 'The effect of thermoxidized oil on hydroxymethylfurfural and acrylamide formation in baked solid model systems'. Then, back to Italy, she started an internship at Agritalia, Naples, where she worked as quality controller of suppliers. In 2009, Teresa started her PhD program at Product design and Quality Management Group (recently renamed to Food Quality and Design) at Wageningen University. The PhD project was part of a broader project entitle "Energy-efficient Drying of Healthy Food Products", aimed to improve the quality of dry foodstuffs focusing on retention of healthy compounds while increasing sustainability of the drying process by reducing the energy consumption. In particular, Teresa studied glucosinolate thermal degradation, myrosinase thermal inactivation and the bioavailability of glucosinolate after consumption of differently processed dried broccoli. During the PhD years, Teresa joined the educational program of the VLAG Graduate School, and she also supervised Master and Bachelor students. Teresa attended several international conferences and she was involved in organising the PhD study tour held in United Kingdom in 2012. Currently she is working as a post-doctoral fellow on Plant Foods to study the effect on meal composition on bioavailability of glucosinolate in broccoli at Food Quality and Design, Wageningen University.

Overview of completed training activities

Courses	Graduate School/Institute	Year
Discipline specific activities		
✓ Reaction kinetics in food science	VLAG	2009
✓ Advanced food analysis	VLAG	2010
✓ Food and Biorefinery Enzymology	VLAG	2011
✓ Microscopy & Spectroscopy in Food and Plant Sciences	EPS	2012
✓ Applied Statistics	WUR	2013
International Congresses		
✓ 28th International Horticultural Congress	IHC	2010
✓ Euro-Mediterranean Symposium for Fruit & Vegetable Processing	INRA	2012
✓ FOOD Denmark PhD congress	Food Denmark	2012
General courses		
✓ VLAG PhD Week	VLAG	2009
✓ Philosophy & ethics of food science & technology	VLAG/WGS	2010
✓ Techniques for writing and presenting a scientific paper	WGS	2010
✓ Project & Time Management	WGS	2011
✓ Career Perspectives	VLAG	2012
Optional		
✓ PhD excursion Australia	FQD	2010
✓ Organizing and participating PhD trip to United Kingdom	FQD	2012
✓ Writing research Proposal	FQD	2013
Teaching		
✓ 20307 Food Processing and Product Properties	FQD	2010

Publications in peer-reviewen journaals

Oliviero T., Verkerk R., Dekker M. (2013). A research approach for quality based design of drying process for retaining health benefits of broccoli. *Trends in Food Science & Technology, 30(2),* 178–184.

Oliviero T., Verkerk R., Dekker M. (2012). Effect of water content and temperature on glucosinolate degradation kinetics in broccoli (*Brassica oleracea* var. *italica*). *Food Chemistry, 132(4),* 2037–2045.

Capuano E., **Oliviero T**., Açarb Ö. Ç., Gökmen V., Fogliano V. (2010). Lipid oxidation promotes acrylamide formation in fat-rich model systems. *Food Research International, 43(4),* 1021–1026.

Oliviero T., Capuano E., Cämmerer B., Fogliano V. (2009). Influence of Roasting on the Antioxidant Activity and HMF Formation of a Cocoa Bean Model Systems**.** *Journal of Agriculture and Food Chemistry, 57 (1),* 147–152.

Submitted articles

Oliviero, T., Verkerk, R., Van Boekel, M.A.J.S. & Dekker, M. The effect of water content and temperature on inactivation kinetics of myrosinase in broccoli (*Brassica oleracea* var. *italica*).

Oliviero T., Jin X., Dekker M., van der Sman R.G.M., Verkerk R., van Boxtel A.J.B. Impact of different drying trajectories on degradation of nutritional components in broccoli.

Oliviero T., Vermeulen M., Verkerk R., Dekker M. *In vivo* formation and bioavailability of isothiocyanates from glucosinolates in broccoli as affected by processing conditions.

Conference abstracts and proceedings

Oliviero T., Verkerk R., Dekker M. The influence of the Adsorption Drying Technology on Stability and Availability of Glucosinolates in Broccoli (*Brassica Oleracea* var. *italica*). 28th International Horticultural Congress, 22-27, Lisbon, August 2010 (Oral Presentation)

Oliviero T., Verkerk R., Dekker M. Effect of moisture content/water activity on thermal degradation of glucosinolates in broccoli (*Brassica oleracea* var. *italica*). Proceedings of the Euro-Mediterranean Symposium for Fruit & Vegetable Processing, Avignon, 18-21 April 2011 (Oral Presentation).

This research was funded by the Energy Research Program EOS of the Dutch ministry of Economics under project EOSLTe7043.

Financial support from Wageningen University for printing this thesis is gratefully acknowledged.

Cover design and layout: Agilecolor Design Studio/Atelier (www.agilecolor.com)
Printed by: Gildeprint Drukkerijen, Enschede (NL) (www.gildeprint.nl)