Glucosinolates during preparation of *Brassica* vegetables in Indonesia

Probo Y. Nugrahedi

Thesis committee

Promotor

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

Co-promotors

Prof. Dr Budi Widianarko
Professor, Food Technology Department
Soegijapranata Catholic University, Semarang, Indonesia

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

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Dr Inge D. Brouwer, Wageningen University
Prof. Dr Katleen Raes, Ghent University, Belgium

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Chapter 1

General Introduction

It is clear that food does not only provide nutrients, but also plays a role in reducing the risk of several diseases. Various studies have been performed associating food intake, especially plant-based foods, with the risk of disease (Shahidi, 2009). For example, meta-analysis studies showed that fruit and vegetable consumption is inversely associated with the risk of coronary heart disease (Dauchet et al., 2006) and cardiovascular mortality (Wang et al., 2014a). Therefore, one of the recommendations for population and individuals to protect and promote health is to increase consumption of fruits and vegetables, legumes, whole grains and nuts (WHO, 2004).

Various components in a plant-based diet are expected to reduce the risk of several chronic diseases. Studies on elucidating bioactive compounds in fruits and vegetables and how they can promote health have been performed. For instance, the antioxidative effect of fruit and vegetables is attributed partially to polyphenols (Quideau et al., 2011). The intake of the polyphenol subgroup of flavonoids associates with a reduced incidence of chronic diseases, such as cardiovascular disease, diabetes, and cancer (Lima et al., 2014; Wang et al., 2014b). The flavonoid kaempferol helps reducing the risk of chronic diseases by enhancing the body's antioxidant defence against free radicals (Chen and Chen, 2013). However, an evaluation by Hollman et al. (2011) showed that the biological relevance of direct antioxidant effects of polyphenols for cardiovascular health could not be substantiated. Although some polyphenol-rich foods exert beneficial effects on some biomarkers of cardiovascular health, there is no evidence that this is caused by improvements in antioxidant function biomarkers.

Another example is the alleged anticancer effect of *Allium* vegetables, berries, and cruciferous vegetables, which is attributed to bioactive compounds, such as organosulfides, polyphenols, phytosterols, β-carotene, α-carotene, and isothiocyanates (Stan et al., 2008). Temple and Gladwin (2003) suggested that the 'teamwork concept', i.e., a wide variety of fruits and vegetables, supplying a wide range of health promoting compounds (phytochemicals), is likely required for reducing the risk for diseases, such as cancer.

The communication of studies supporting the vital role of diet to promote health has increased the interest of consumers in consuming food for health (Rodriguez et al., 2006), including in Indonesia (Basu et al., 2007). Studies on revealing the occurrence and mode of action of health promoting compounds, e.g., carotenoids, phenolics, and flavonoids, in plant-based foods as well as in locally grown fruit and vegetables in Indonesia have been performed (e.g., Zakaria-Rungkat et al., 2000; Setiawan et al., 2001; Andarwulan et al., 2010).

However, as most vegetables are processed and/or prepared before consumption, the effects of preparation on the phytochemicals should be taken into consideration. For instance, Kalt (2005) reported that various processing methods, such as blanching, microwave processing, boiling, and stir frying, can change and often damage the antioxidants in fruit and vegetables resulting from, e.g., oxidation, thermal degradation, and leaching, especially for vitamin C and phenolics. Meanwhile for carotenoids, processing can also lead to a dissociation of antioxidants from plant matrix components and improved digestive absorption (Kalt, 2005).

Ruiz-Rodriguez et al. (2008) comprehensively reviewed the effect of domestic processing on bioactive compounds in food. Non water-soluble compounds, such as carotenoids, tocopherols, and polyunsaturated fatty acids, are less affected by cooking methods involving water than those using oils. Moreover, tocopherols and carotenoids become more available with blanching or steaming due to the destruction of cell wall material in which they are located. However, for more polar compounds, such as phenolics, folates, and glucosinolates, the losses due to water cooking methods are more profound, particularly due to leaching in addition to thermal degradation. Among other methods, steaming can maintain the content of these compounds (Ruiz-Rodriguez et al., 2008).

Brassica **vegetables and health**

Brassica vegetables, e.g., broccoli, cauliflower, red cabbage and Brussels sprouts, have been widely investigated for their beneficial effects on human health. Studies reported significant constituents in the vegetables, including vitamins, minerals, phenolic compounds, fibres, and carotenoids. Moreover, these different groups of phytochemicals contribute to the reported antioxidative, anticarcinogenic, and cardiovascular protective effects of *Brassica* vegetables (Jahangir et al., 2009). A special group of phytochemicals that is present almost exclusively in *Brassica* is the group of glucosinolates (Bellostas et al., 2007).

Epidemiological studies reported inverse association between *Brassica* vegetables intake and the risk of certain cancers (Voorrips et al., 2000a; Voorrips et al., 2000b; Higdon et al., 2007). Herr and Büchler (2010) suggested that these vegetables contain chemo-preventive agents against lung, colorectal, breast, prostate, pancreatic, and possibly also gastric cancers. Glucosinolate (GS) intake is expected to lower the risk of cancer (Verhoeven et al., 1997; Cirignano and Morgan, 2014). Epidemiological research on *Brassica* vegetable intake and lung cancer has provided strong evidence that GSs in these vegetables are precursors of chemopreventive compounds (Brennan et al., 2005). A recent review by Eid et al. (2014) showed that GSs intake in *Brassica* spp is associated with the reduction in colorectal cancer risks.

The breakdown products of GSs, particularly isothiocyanates (ITCs), have been reported to act on the process of carcinogenesis by influencing several phases of tumour initiation, promotion and progression, and by suppressing the final steps of carcinogenesis. Several mechanisms of the chemoprotective effect of ITCs include modulation of xenobiotic metabolism, increasing the antioxidant capacity of cells, and inhibition of tumour growth. During carcinogen metabolism ITCs are involved in inhibiting the metabolic activation of carcinogens by cytochrome P450 (phase 1) and inducing phase 2 detoxifying enzymes and cellular defensive enzymes. For oxidative stress protection, ITCs indirectly act to increase the antioxidant capacity by either inducing phase 2 enzymes or by increasing glutathione levels. The mechanisms for cytostatic and cytotoxic effects of ITCs consist of the induction of apoptosis, inhibition of cell cycle progression and of angiogenesis (Traka and Mithen, 2009; Molina-Vargas, 2013).

The healthiness of a *Brassica* product can be partly established by monitoring the GSs and their breakdown and correlating this with biological activities (Verkerk et al., 1997). And since the GS breakdown is strongly influenced by the enzymatic activity of myrosinase, the interaction between GS and myrosinase during processing must be taken into account.

Glucosinolates-Myrosinase system

Glucosinolates (GSs) are a group of plant secondary metabolites, structurally contain β-thioglucoside N-hydroxysulphates with a sulphur linked β-D-glucopyranose moiety and variable side group (R). Based on this side chain, GSs are usually classified into aliphatic, aromatic, and indole GSs. Aliphatic GSs derive from methionine, indole GSs from tryptophan, and aromatic GSs mostly derive from phenylalanine. Table 1.1 shows the chemical names and structures of the main GSs from the *Brassica* species. The biosynthesis of GS proceeds through three separate biosynthetic steps, i.e., 1) chain elongation of selected precursor amino acids, 2) formation of the GS core structure, and 3) modifications of the side chain (Hanschen et al., 2014).

In plants, GSs are commonly involved in the plant's defence system to stress. In intact plant tissue, GSs are stored in compartments separated from the endogenous enzyme myrosinase (thioglucoside glucohydrolase EC 3.2.1.147). GSs are highly prone to degradation initiated by myrosinase-catalysed hydrolysis upon cell disruption (Fahey et al., 2001; Verkerk et al., 2009; Hanschen et al., 2014).

Table 1.1. Chemical names and structures of the main glucosinolates in *Brassica* vegetables

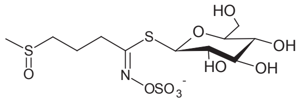
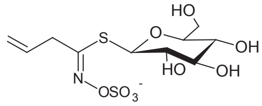
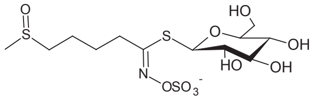
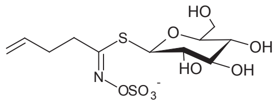
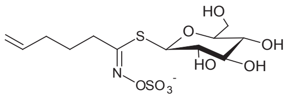
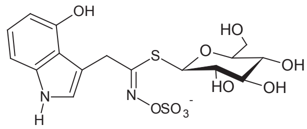
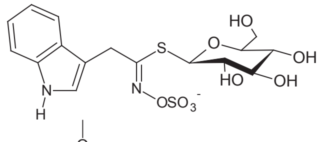
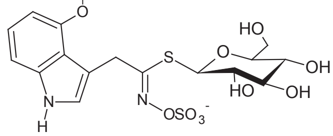
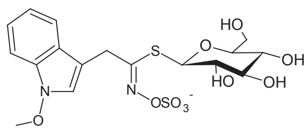
Trivial name	Chemical name	Chemical structure
Glucoiberin	3-Methylsulfinylpropyl-GS	
Sinigrin	Prop-2-enyl-GS	
Glucoraphanin	4-Methylsulfinylbutyl-GS	
Gluconapin	But-3-enyl-GS	
Glucobrassicanapin	Pent-4-enyl-GS	
4-Hydroxy-glucobrassicin	4-Hydroxy-indol-3-ylmethyl-GS	
Glucobrassicin	Indol-3-ylmethyl-GS	
4-Methoxy-glucobrassicin	4-Methoxy-indol-3-ylmethyl-GS	
Neoglucobrassicin	N-methoxyindol-3-ylmethyl-GS	

Figure 1.1 shows the enzymatic breakdown of GSs. Due to the activity of myrosinase the GS will degrade into glucose and an unstable aglycon intermediate, thiohydroxamate-*O*-sulfonate. The unstable aglycon rearranges into different breakdown products, including ITCs, thiocyanates, nitriles, and epithionitriles, depending on GSs substrate, pH, presence of Fe^{2+}, or epithiospecifier protein (Mithen et al., 2000; Fahey et al., 2001).

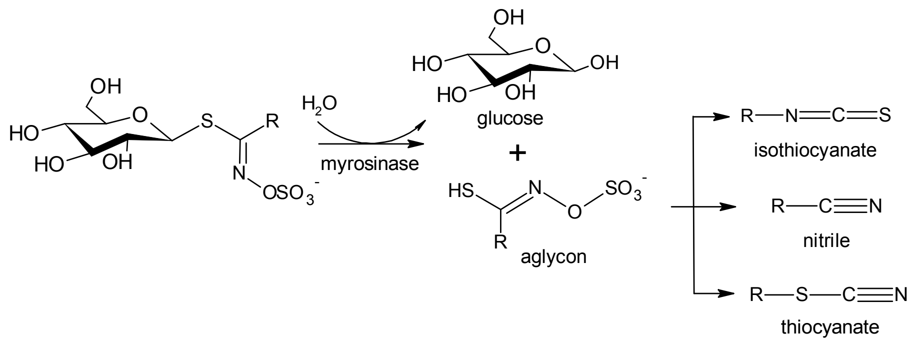


Figure 1.1. Enzymatic breakdown of glucosinolates

Inactivation of myrosinase, therefore, can reduce the ITCs formation. Fortunately, a myrosinase-like activity is also provided by the microflora in the large-intestine. Ingestion of GS-contained products without active plant myrosinase still leads to formation and absorption of bioactive breakdown products by enzymes from the gut flora, although their bioavailability is lower than the ones with active plant myrosinase (Conaway et al., 2000; Higdon et al., 2007; Traka and Mithen, 2009; Oliviero et al., 2014b).

Myrosinase is a thermolabile enzyme, the activity of which is considerably reduced at moderately high temperatures. Furthermore, the heat stability of myrosinase is shown to be dependent on its source (Rungapamestry et al., 2007). The temperatures applied during processing of *Brassica* vegetables quickly inactivate myrosinase (Ludikhuyze et al., 1999; Rungapamestry et al., 2006; Verkerk et al., 2010; Hennig et al., 2013). Different optimum temperatures for the activity of myrosinase from *Brassica* vegetables were also reported (Springett and Adams, 1989; Yen and Wei, 1993; Ludikhuyze et al., 2000). The activity of myrosinase is also influenced by pH (Springett and Adams, 1989; Yen and Wei, 1993; Ludikhuyze et al., 2000; Van Eylen et al., 2008), the presence of ascorbic acid, salt, and pressure (Ludikhuyze et al., 2000; Suzuki et al., 2006; Van Eylen et al., 2008).

Effect of processing

Brassica vegetables are mainly consumed after processing, e.g., boiling, steaming, microwave processing, stir-frying, or fermentation. Food preparation, particularly that involving heat, is aimed to inactivate pathogens, to increase sensorial liking and digestibility, and other beneficial changes, but it can also lead to negative impacts, such as losses of certain (micro)nutrients and phytochemicals, formation of toxic compounds or of compounds with negative effects on sensorial perception (van Boekel et al., 2010). Previous studies reported that different preparation techniques performed on *Brassica* vegetables could affect sensorial acceptance in various ways (Poelman and Delahunty, 2011; Poelman et al., 2013). Unfortunately, processing usually leads to an adverse impact on the health promoting compounds, including the GS, polyphenols and ascorbic acid (Lee and Kader, 2000; Vallejo et al., 2002; Galgano et al., 2007; Podsedek, 2007; Borowski et al., 2008; Miglio et al., 2008; El Gharras, 2009; Pellegrini et al., 2010). For example, Gliszczyñska-šwiglo et al. (2006) reported that cooking of broccoli in water reduced the concentrations of flavonoids, phenolic acids, GS, and vitamin C. Vallejo et al. (2002) showed that high pressure and conventional boiling had a significant loss of total GSs (33% and 55%, respectively) in broccoli, whereas steaming had minimal effects on GSs and vitamin C. The authors also reported that microwave processing caused loss of vitamin C at 40% and total GSs at 74% in broccoli (Vallejo et al., 2002). However, Verkerk and Dekker (2004) found that microwave processing did not decrease the GSs in red cabbage. Differences in experimental setup could be the reason for this discrepancy. Fermentation also reduced total GS concentration substantially (Daxenbichler et al., 1980; Ciska and Pathak, 2004). Among other processing methods, steaming can be considered as the method that best preserves health-promoting compounds.

***Brassica* vegetable processing in Indonesia**

Indonesia produces large amounts of *Brassica* vegetables, among other vegetables, which comprises of various locally grown species (Siemonsma and Piluek, 1993). Total production number of cabbages and other *Brassicas* from 2009 to 2013 is more than 1.3 million tonnes per year (FAOSTAT, 2014; Statistics-Indonesia, 2014).

Vegetables are prepared into various dishes and are consumed on a daily basis. In urban areas of Indonesia, the role of food service establishments in providing daily meals to people is important. Food service establishments, from restaurants to street food vendors, play a vital role in supplying food with a continuous availability, affordable price, and convenience for the consumers. Besides that, domestic preparation by household members still plays a vital role in providing the meals at home.

In the Southeast Asian cuisine, preparation of the ingredients, such as peeling, cutting/ slicing/ chopping, and grinding, requires a lot of time, but in general the preparation or cooking time is short (Van Esterik, 2008). Preparation steps within the Indonesian vegetables production chain have different characteristics, for example stir-frying, cooking with extensive amount of water, salting and fermenting, and re-heating. Stir frying is one of the most popular methods to prepare vegetables. Cooking vegetables also commonly involves the addition of spices, garlic, chili, salt, sugar and etc. as ingredients, for getting the optimum sensorial quality.

White cabbage, for example, is often subject to thermal processing, such as boiling, steaming, and stir frying, prior to consumption. The dishes are various, ranging from soup (e.g., *sup* and *sayur asam*/ tamarind vegetable soup) to stir fried (e.g., *cap cay*/ chop suey and *ca*). *Brassica* vegetables are also served as minor part of main dishes, such as any types of noodles, vermicelli, snacks, meatball soup, etc.

One local product, namely a steamed cabbage roll, is made by blanching the leaf layers of cabbage followed by rolling and steaming. Steaming of the cabbage roll is usually employed in one steamer as a kind of dim sum, locally called *siomay*, which includes, for instance, boiled potato, bitter melon, tofu, and egg (Figure 1.2, left). This dish is usually served with peanut butter sauce. When sold as street food, the food can be steamed either constantly for more than two hours, depending on the selling rate, on a very low flame level. By some vendors after preparation, the rolls are stored at ambient temperature and only steamed for some minutes in order to reheat the food.

An example of a fermented product is *sayur asin* (Figure 1.2, right). Similar products, with own local names, have been also produced in other Asian countries (Lee, 1997). *Sayur asin* is prepared by naturally fermenting Indian mustard (*Brassica juncea*) in the presence of brine. Either the starch liquid from boiling rice (local name: *tajin*) or coconut water is used as the medium of *sayur asin* fermentation.

Obviously, there exists a striking difference in commonly practiced preparation methods between Western and Southeast Asian countries. Therefore, it is of interest to study and compare the cooking methods in relation to the underlying molecular mechanisms such as cell lysis, diffusion, thermal degradation, and leaching of GSs and the consequences for the GS profiles and concentrations. Based on these mechanisms, mathematical models can be applied to describe the changes of concentration of GSs as affected by processing in a quantitative way. Different types of preparation of *Brassica* vegetables affect the magnitude of impact on the content of health promoting compounds. Recently, Hanschen et al. (2014)

reviewed the reactivity and stability of GSs and their breakdown products in foods. They showed that GSs and ITCs in *Brassica* vegetable-based food are subject to a variety of changes during food processing.

Figure 1.2. *Siomay* (left) and *sayur asin* (right)

Mathematical modelling approach

Processing is commonly applied to vegetables prior to consumption. The effects of processing on the behaviour of GS content in *Brassica* vegetables can be described quantitatively by capturing the information in the experimental data, employing parameter estimation, into a mathematical model. Kinetic modelling has been used before to study the effect of food processing on the concentration of health-promoting compounds, including GSs (Oerlemans et al., 2006; Dekker et al., 2009; Hennig et al., 2012; Sarvan et al., 2014). A model attempts to formulate the behaviour of a system based on knowledge of the properties of the component parts. More specifically, kinetic modelling is a mathematical tool to describe and predict changes in food properties (Van Boekel, 2009).

Such a model can be used to simulate scenarios, by changing the parameter(s), to predict as well as to optimise the GS content in the product. The model can also be used to design or modify the preparation method to produce the optimum GS content in a *Brassica* product. Thus, the use of a mathematical model describing the different mechanisms that can affect quality attributes is essential to design, optimise, and predict the quality of foods (Van Boekel, 2009).

Objective and outline of the thesis

The actual concentrations of GSs in *Brassica* vegetables at the moment of consumption are highly variable. Many steps in the food production chain, including processing of *Brassica* vegetables can have a large impact on the GS content, myrosinase activity, and therefore, on the health promoting effect (Jones et al., 2006; Verkerk et al., 2009; Sarvan et al., 2012; Bongoni et al., 2014). Consequently, it is impossible to convert data on intake of *Brassica* vegetables into accurate data on intake of GSs.

Different conditions in processing *Brassica* vegetables are hypothesized to lead to various degrees of impact to the GS concentration, which eventually give a significant influence on the actual intake of GSs. In the case of Indonesia, there is no study on the effects of typical Indonesian preparation methods on GS content in *Brassica* vegetables. The general objective of this thesis is to investigate the GS content of *Brassica* vegetables upon consumption by considering the various steps in the production chain and to investigate whether it is possible to optimize the GS content. Thus, the work presented in this thesis focuses on the following research questions:

1. How do food handlers of food service establishments and households (in Indonesia) prepare *Brassica* vegetables and what is their perception on health effects upon the preparation method and *Brassica* type?
2. How does time-temperature of *Brassica* vegetable preparation, particularly steaming and stir-frying, affect the GS content?
3. What is the effect of myrosinase activity on GS content during fermentation of *Brassica* vegetables?
4. What is the relative importance of underlying mechanisms in the preparation method affecting the changes of GS content?

This thesis aims to study the effects of some typical Asian (Indonesian) preparation methods, i.e., fermentation, steaming, and stir frying, on the GS content of *Brassica* vegetables. In order to do this, a mechanistic perspective that underlies the GS changes during preparation will be employed. This study complements other PhD projects on (1) genetic effects related to GS degradation during food processing to breed vegetables with improved GS retention (Hennig, 2013), (2) the effect of temperature and water content during air drying on GS degradation and myrosinase inactivation, and on the absorption of ITCs of dried product after consumption (Oliviero, 2013), (3) consumer behaviour (in this case: Dutch households) towards vegetables and optimising domestic processing conditions applied by consumers on the sensory and health properties (Bongoni, 2014), and (4) on mathematical modelling on thermal treatment of *Brassica* (Sarvan et al., 2012; Sarvan et al., 2014). The results are laid down in the following chapters.

In ***Chapter 2*** the reported changes of GSs in *Brassica* during various preparation methods are reviewed from literature. Accordingly, the molecular mechanisms underlying these changes of GS concentration are proposed. The need for further exploration on specific Eastern preparation methods of *Brassica* vegetables is underlined and explored in the following chapters.

To obtain information and knowledge on how the vegetables are commonly prepared ***Chapter 3*** describes the results of a survey on *Brassica* vegetable preparation performed by food handlers in food service establishments and households in Semarang city, Indonesia. Moreover, their perception of health on both the vegetables and the preparation methods is presented. The consequences of the various applied preparation methods on the content of alleged health promoting GSs in dishes containing *Brassica* vegetables are discussed by integrating the survey data with knowledge on the mechanisms of changes in the content of GSs for identifying the technological and nutritional implications.

Chapter 4 studies the effect of a long duration of steaming on the GS content, colour, and texture of the cabbage roll. The changes of GSs during steaming are mathematically modelled to gain insight in the important mechanisms involved. Subsequently, ***Chapter 5*** studies the fate of GS concentration during production of a fermented local product made from *Brassica juncea*, i.e., *sayur asin*, and the effect of myrosinase activity on the GS concentration during fermentation.

Stir frying is one of the most familiar techniques to prepare *Brassica* vegetables in Indonesia. ***Chapter 6*** describes how the GS profiles in two *Brassica rapa* vegetables, i.e., Chinese cabbage (*Brassica rapa* ssp. *pekinensis*) and pakchoi (*Brassica rapa* ssp. *chinensis*), are affected by stir frying at various times and temperatures. Finally, ***chapter 7*** discusses all these findings and elaborates them on the basis of underlying mechanisms on the GS changes during processing.

Chapter 2

A mechanistic perspective on process-induced changes in glucosinolate content in *Brassica* vegetables : a review



Probo Y. Nugrahedi, Ruud Verkerk, Budi Widianarko, and Matthijs Dekker
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Abstract

Brassica vegetables are consumed mostly after processing, which is expected to give beneficial effects on the vegetable properties, such as improved palatability and bioavailability of nutrients, or shelf life extension. But, processing also results to various changes in the content of health promoting phytochemicals like glucosinolates. This paper reviews the effects of processing on the glucosinolate content by using a mechanism approach underlying processing method employed. Cultural differences between Eastern and Western preparation practices and their possible effect on glucosinolate retention are highlighted. Boiling and blanching considerably reduce the glucosinolate content mainly due to mechanisms of cell lysis, diffusion, and leaching, and partly due to thermal and enzymatic degradation. Steaming, microwave processing, and stir-frying either retain or slightly reduce the glucosinolate content due to low degrees of leaching; moreover, these methods seem to enhance extractability of glucosinolates from the plant tissue. Fermentation reduces the glucosinolate content considerably, but the underlying mechanisms are not yet studied in detail. Studying the changes of glucosinolates during processing by a mechanistic approach is shown to be valuable to understand the impact of processing and to optimize processing conditions for health benefits of these compounds.

Keywords glucosinolate, *Brassica* vegetable, processing, mechanistic approach, health

Introduction

Vegetables of *Brassica* spp, such as white and red cabbages, broccoli, cauliflower, and Brussels sprouts have been studied extensively in particular for their health beneficial properties. These vegetables contain specific health promoting compounds, i.e., glucosinolates (GSs), which distinguish them from other vegetables in the human diet (Verhoeven et al., 1996; Fahey et al., 2001). In addition, *Brassica* vegetables also contain significant levels of other health promoting compounds, such as polyphenols, carotenoids, tocopherols, and vitamin C (Kurilich et al., 1999; Borowski et al., 2008).

Brassica vegetables are mainly consumed after some types of processing, e.g., boiling, steaming, microwave processing, stir-frying, or fermentation. The combination of procedures and ingredients to process *Brassica* vegetables differs between regions. For example, in many Southeast Asian countries stir-frying is a popular quick cooking methods for the vegetables (Van Esterik, 2008). Various preparation and processing methods and conditions can also be found for boiling, steaming, and fermenting of *Brassica* vegetables.

Processing is one of the major factors affecting changes of GS content along the production chain (Dekker et al., 2000). Various reviews have briefly discussed the effects of processing on GS content (e.g. Fenwick and Heaney, 1983; Mithen et al., 2000; McNaughton and Marks, 2003; Jones et al., 2006; Higdon et al., 2007; Cartea and Velasco, 2008; Traka and Mithen, 2009; Verkerk et al., 2009). McNaughton and Marks (2003) predicted that average loss of GSs in *Brassica* during processing is approximately 36%. More recently, Ruiz-Rodriguez et al. (2008) summarized the effects of some domestic processing methods on GS content qualitatively and indicated steaming is the best method retaining the GS content. Moreover, Rungapamestry et al. (2007) reviewed the effects of boiling, blanching, steaming, and microwave processing on GS content, myrosinase activity, and the formation and bioavailability of GS breakdown products. The authors, however, indicated complexities to compare and interpret data directly due to variability of parameters and differences in processing conditions or analytical methods. Moreover, previous research on the processing effects of *Brassica* vegetables on GS content, sometimes showed inconsistent or conflicting results between different studies (López-Berenguer et al., 2007; Rungapamestry et al., 2007; Song and Thornalley, 2007; Francisco et al., 2010; Pellegrini et al., 2010).

Processing changes GS content through several mechanisms, such as enzyme-catalysed breakdown, thermal breakdown, cell lysis, and leaching (Dekker et al., 2000). Each processing method involves specific conditions, which lead to various degrees of impact of the different mechanisms on the GS content. Using the underlying mechanisms that are critical for a processing method can be a valuable approach to understand the kinetics of GS changes. In this paper the health related properties of GS in *Brassica* vegetables that have been reviewed extensively (e.g. Mithen et al., 2000; Traka and Mithen, 2009; Verkerk et al., 2009; Herr and Büchler, 2010) will only be briefly discussed. The paper focusses on the review of the effects of processing on the GS content in *Brassica* vegetables currently published and analyses these changes of GS by discussing the relevant mechanisms for each processing method. It is shown that different conditions in processing *Brassica* vegetables can have a significant influence on the final intake of GSs. Furthermore, the value of a mechanistic approach in future research on process-induced changes in GSs is discussed.

Health promoting glucosinolates in *Brassica* vegetables

Epidemiological cohort studies have reported inverse associations between intake of *Brassica* vegetables and the risk of lung, colon, and rectal cancers (Voorrips et al., 2000a; Voorrips et al., 2000b). More recently, based on epidemiological evidence reports Herr and Büchler (2010) suggested that these vegetables contain chemopreventive agents against lung, colorectal, breast, prostate, pancreatic, and possibly also gastric cancers. The GS content of *Brassica* vegetables is assumed to be accountable indirectly to lower the risk of cancer (Verhoeven et al., 1997). Isothiocyanates (ITCs) are one of the GS breakdown products, which have a vital role to reduce the risk of cancer by inhibiting phase 1 and inducing phase 2 enzymes during carcinogen metabolism. ITCs act on the process of carcinogenesis by influencing phases of tumor initiation, promotion and progression, and by suppressing the final steps of carcinogenesis (Traka and Mithen, 2009).

GSs are a group of water-soluble secondary plant metabolites. Structurally a GS comprises β-thioglucoside N-hydroxysulphates with a sulphur linked β-D-glucopyranose moiety and side-chain group (R) (Figure 2.1). The side-chain structure is highly diverse, derived mainly from one of certain amino acids, such as methionine, tryptophan, and phenylalanine. The sulphate group is normally balanced by a (potassium) cation. The GS can be classified either as aliphatic, aromatic, or indole (Mithen et al., 2000; Verkerk et al., 2009).

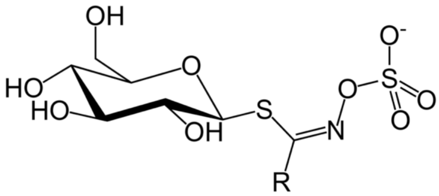

Figure 2.1. General structure of glucosinolate (R is the variable side chain)

GSs are stored in tissue compartments that are physically separated from compartments containing myrosinase enzyme (thioglucosidase EC 3.2.1.147) in intact plant tissue. Upon tissue damage GSs are highly prone to hydrolytic degradation catalysed by myrosinase into glucose and an unstable aglycon intermediate, thiohydroxamate-*O*-sulfonate. The unstable aglycon rearranges into different breakdown products, including ITCs, thiocyanates, nitriles, and epithionitriles, depending on GS substrate, pH, presence of Fe^{2+}, or epithiospecifier protein (Mithen et al., 2000; Fahey et al., 2001). Moreover, GSs can also be degraded thermally and chemically (MacLeod et al., 1981; Chevolleau et al., 1997; Bones and Rossiter, 2006).

Myrosinase, consequently, has an essential role on the GS conversion to ITCs. The activity of myrosinase is influenced by some intrinsic and extrinsic factors, such as ascorbic acid, $MgCl_2$, pH, temperature, and pressure (Ludikhuyze et al., 2000). Inactivation of myrosinase, therefore, substantially decreases the bioavailability of ITCs (Higdon et al., 2007). Fortunately, a myrosinase-like activity is also provided by the microflora in the gastrointestines. Ingestion of GS-contained products without active plant myrosinase still leads to formation and absorption of bioactive breakdown products by enzymes from the gut flora, although their bioavailability is lower than the ones with active plant myrosinase (Conaway et al., 2000; Higdon et al., 2007; Traka and Mithen, 2009).

The GS content in *Brassica* vegetables prior to consumption is highly diverse due to variation in the steps along the food supply chain, including breeding, cultivation, storage and packaging, and processing (Verkerk et al., 2009). Dekker et al. (2000) predicted that changes in GS content in *Brassica* vegetables may vary by 5–10 fold at critical steps in the production chain due to genetic and environmental variation, postharvest handling, packaging and storage, and processing condition. The resulting GS content at the end of the chain have been shown to vary over 100 fold as a result of variations in the supply chain conditions (Dekker and Verkerk, 2003). During digestion, the fate of GSs could be further influenced by the extent of cell rupture, gastrointestinal transit time, meal composition, individual genotype, and variation in colonic microflora (Rungapamestry et al., 2007).

Mechanisms underlying glucosinolate changes during processing

Prior to preparation *Brassica* vegetables are subjected to postharvest treatments, including packaging and storage, washing, and cutting or chopping. These stages generally can reduce the GS content (Jones et al., 2006; Rungapamestry et al., 2007; Song and Thornalley, 2007; Verkerk et al., 2009). These reductions are likely caused by cell and tissue damage, leaching, and myrosinase activity. However, Verkerk et al. (2001) reported that storing of chopped cabbages and broccoli induced physiological responses, similar to insect damage, and consequently, increased especially the indole GS content. In general, the effects of postharvest treatments and storage are usually less pronounced than the changes induced by processing and preparation.

Vegetable tissues, cells, and cellular compartments are further damaged during processing and preparation. Degree of disruption and subsequent changes of GS content depend on the nature of the vegetables and the processing methods. Both domestic and industrial processing types are normally applied on *Brassica* vegetables. It is likely that the domestic processing method is strongly influenced by local culture and preferences on the final products. One normally considers sensorial properties, i.e., taste, texture, and colour of the expected products, subjectively, as the critical quality attributes to decide time-temperature settings of the domestic cooking process. Consequently, this variability in the process conditions will lead to variability of the GS content in the final products.

Most of processing methods on *Brassica* vegetables apply heat, which is transferred into the plant tissue by a heat-transfer medium, e.g., steam, water, or cooking oil. Dekker et al. (2000) and Volden et al. (2008b) have proposed a model of GS breakdown during boiling of *Brassica* vegetables. There are either sequential or simultaneous mechanisms taking place during boiling, which involve (bio)chemical reactions, heat and mass transfer (Figure 2.2 and Table 2.1), namely:

1. lysis of cells and cellular compartments,
2. diffusion of components through the lysed tissue,
3. enzyme-catalysed hydrolysis of GSs, is possible upon lysis and diffusion (in cooking water and/or in lysed vegetable tissue),
4. thermal degradation of GSs (and further degradation of their breakdown products),
5. inactivation of myrosinase as well as loss of enzymatic co-factors, such as ascorbic acid, Fe^{2+}, epithiospecifier protein, or thiocyanate-forming protein, and
6. leaching of GSs and breakdown products into boiling water after lysis and diffusion.

The changes in GS content during processing can be explained by these mechanisms, depending on the processing conditions like the temperature-time profile. The mechanisms (1) to (6) can be captured in (differential) equations containing parameters describing the rate constants of the different mechanisms (see e.g. Volden et al., 2008b; Sarvan et al., 2012). Also the effect of temperature on these rate constants can be captured by a mathematical model. It has been shown that the rate constants of thermal degradation are different between specific GS (Oerlemans et al., 2006). Indole GSs generally react faster than aliphatic GSs. The variety of the *Brassica* vegetables, moreover, also affects the thermal degradation rate constant of each GS. So, the reaction rate is depending both on the type of GS and also on the plant matrix that it is present in (Dekker et al., 2009; Hanschen et al., 2012).

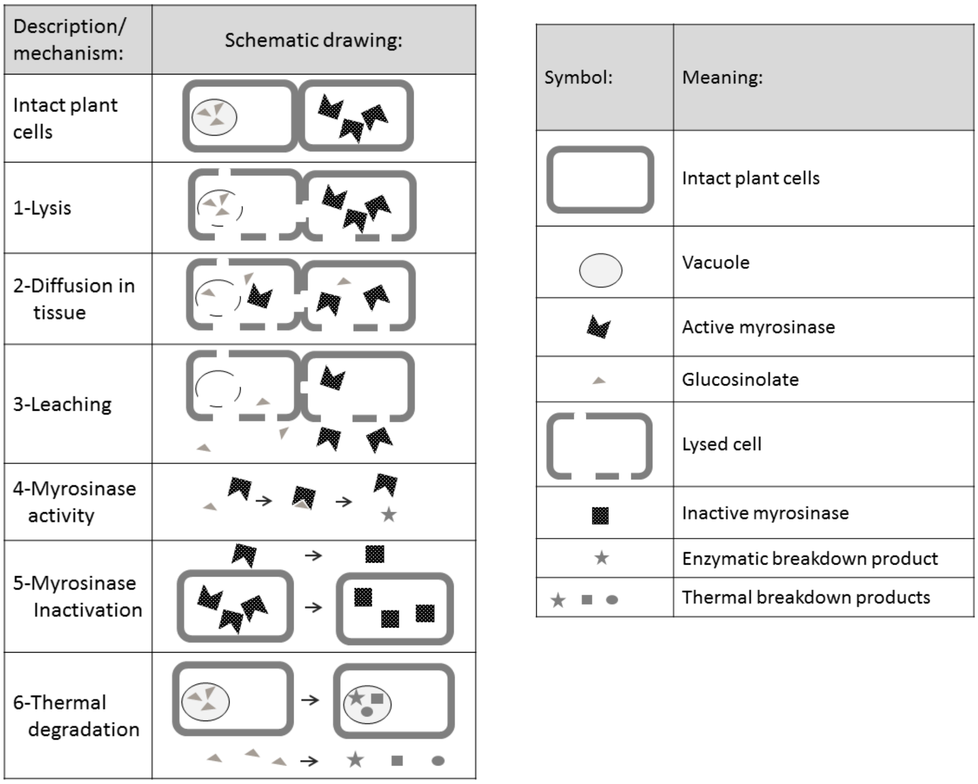

Figure 2.2 a. Schematic illustration of the main mechanisms responsible for the changes in glucosinolate content during *Brassica* vegetable preparation
b. Legend to explain the used symbols.

Table 2.1. Relative importance of the main mechanisms involved in changing the glucosinolate content during different preparation methods

Mechanism	Preparation method				
	Boiling	Steaming	Blanching	Microwave processing	Stir-frying
1. Lysis	+	+	+	+	+
2. Diffusion in tissue	+	+	+	+	+
3. Leaching	+	+/-	+/-	+/-	-
4. Myrosinase activity	-	+/-	-	-	-
5. Myrosinase inactivation	+	+	+	+	+
6. Thermal degradation	+/-	+/-	+/-	+/-	+

Boiling

Boiling is probably the most frequent processing method applied on *Brassica* vegetables. This is employed simply by immersing the vegetable into cold or already boiling water. Heat is transferred mainly by convection of hot water into the vegetable tissue until a final temperature is reached at about 100 °C. Boiling is continued for a certain time, depending on the type of *Brassica* and the sensory preferences of the consumer. During the heating of the vegetable tissue, cell lysis will gradually occur. Cell and cell organelle membranes will collapse and cell walls will soften. Consequently, both GSs and the myrosinase enzyme will diffuse through the lysed tissue and also partly leach into the cooking water. Enzymatic breakdown of GSs can occur both in the damaged tissue and in the cooking water. At the same time, however, enzyme denaturation can occur due to the high temperature. Enzymatic breakdown is usually expected to be limited, depending on the rate of temperature rise of the vegetable tissue and the stability of the specific myrosinase. Thermal (non-enzymatic) degradation of GSs can occur at boiling temperature. Depending on the process conditions, the type of GS, and the type of *Brassica* vegetable, losses of 5–20% of GSs due to thermal breakdown are typically expected for boiling processes (Oerlemans et al., 2006; Dekker et al., 2009; Jones et al., 2010).

Leaching is the major factor of GS loss during boiling of vegetables. It is strongly affected by the ratio of vegetable to water, the boiling time and method, and by the type and geometrical shape of the vegetable tissues (Rosa and Heaney, 1993; Dekker et al., 2000; Volden et al., 2008a). Losses of 25%–75% of GSs due to leaching are typically expected for boiling processes, depending on the process condition, the type of GS, and the type of *Brassica* vegetable. In Table 2.2, the published results on GS retention upon boiling are shown. Rungapamestry et al. (2008) reported a small loss, yet statistically significant, of total GSs in broccoli after boiling, which is most likely due to the short boiling time employed. Pellegrini et al. (2010) accordingly found that boiling retained total GS content in broccoli and Brussels sprouts but, reduced total GS content in white cauliflowers. They reported, furthermore, a high degree of total GS loss when blanch-freezing was employed prior to boiling. The prior processing could enhance the disruption of vegetable tissue to cause extensive cell lysis, diffusion, and subsequent leaching during the boiling of the damaged tissue. In contrast, D'Antuono et al. (2007) reported that total GS content was two-fold higher in boiled cauliflower compared to raw. The authors suspected that this was due to higher extractability of GSs in the boiled than in the raw cauliflower tissues. Higher degree of GS loss in broccoli was reported when boiling was employed at higher pressure than normal pressure (Vallejo et al., 2002). However, Francisco et al. (2010) found no differences of aliphatic, indole, and total GS contents in turnips greens boiled at either normal or high pressure.

Previous studies showed that the main parts of leached GSs were recovered in the cooking water (Rosa and Heaney, 1993; Song and Thornalley, 2007; Volden et al., 2009b; Francisco et al., 2010). Meanwhile, the unaccountable loss was likely due to thermal degradation and myrosinase-catalysed hydrolysis (Rungapamestry et al., 2007; Volden et al., 2009b). Song and Thornalley (2007) found that boiling for 30 min retained the sum of GSs from boiled vegetable and cooking water. However, Ciska and Kozłowska (2001) reported that degree of leaching of GSs in cabbage during boiling for 5–25 min was steady at about 10% of the GSs in raw material. The rest of the loss was unaccounted for but, it is most likely caused by thermal breakdown both in the plant tissue and in the water as it increased during boiling. In accordance, Kassahun et al. (1996) reported a higher degree of unaccountable loss of GSs than leaching during boiling of cabbage for 60 min in acidic solution.

To summarize, boiling generally reduce GS content of *Brassica* vegetables mainly due to the extensive leaching following cell lysis and diffusion. However, inconsistent or conflicting results are sometimes reported which might be due to the effect of heating on the extraction efficiency of GSs.

Table 2.2. Effect of boiling on glucosinolate retention

Brassica	Temperature (°C)	Time (min)	Size	*Brassica* : Water (w/v or w/w)	GS Retention (%)[1] Aliphatic	Indole	Aromatic	Total	References
Broccoli	NA	10-15	NA	NA	41.5	58.1	NC	44.2	(Cieslik et al., 2007)
	Boiling water	30	Floret	1:5-7.5	17.0	NA	50.0	19.4	(Song and Thornalley, 2007)
		8	Floret + stem 2.5 cm	1:5	126.9	70.5	NA	95.3	(Pellegrini et al., 2010)
		15	Floret + stem (initially frozen)		58.5	20.4	NA	35.9	
		3	Floret 40 mm length	1:5	126.6	76.1	44.0	90.5	(Rungapamestry et al., 2008)
		3 (& kept for 2 h)			103.9	50.3	20.0	65.7	
		NA	Floret + stem 25 mm	1:5	126.3	38.7	NA	41.0	(Miglio et al., 2008)
		5	Floret	1:3	54.2	51.7	50.0	53.5	(Gliszczyñska-świglo et al., 2006)
	NA	5	Floret 3 cm dim. + stem 1 cm	1:1	56.8	18.4	UP	25.5	(Vallejo et al., 2002)
	High pressure	3			13.6	54.3	NC	47.6	
	Boiling water	2; 5	Floret (2 cultivars)	1:2	70.3-85.5	71.3-97.5	NA	70.4-88.1	(Jones et al., 2010)
		5	Piece	1:2	58.8	40.7	NA	53.5	(Yuan et al., 2009)
Brussels sprouts	NA	10-15	NA	NA	40.9	30.6	NC	36.8	(Cieslik et al., 2007)
	Boiling water	30	Whole	1:5-7.5	41.9	NA	37.7	41.6	(Song and Thornalley, 2007)
		10	Whole	1:5	102.0	105.5	NA	103.9	(Pellegrini et al., 2010)
		7	Whole (initially frozen)		49.4	70.9	NA	59.3	
Cabbage		10	30-40 cm^2 (3 cultivars)	1:5	41.7-47.1	31.4-46.1	NA	39.5-46.9	(Rosa and Heaney, 1993)
		5-30	Strips 5 mm thick	1:3	14.4-66.0	11.6-66.0	NC-50.0	13.3-65.9	(Ciska and Kozłowska, 2001)

		30	Whole or largely cut	1:5-7.5	34.8	NA	NC	34.8	(Song and Thornalley, 2007)
	Boiling water; cold start	15; 30; 60	Slice	1:1	NA	NA	NA	1.3-27.8	(Kassahun et al., 1996)
Red cabbage	Boiling water	10	1 x 1 cm	1:1	60.1	57.7	71.6	62.4	(Volden et al., 2008a)
Cauliflower		30	Floret	1:5-7.5	24.2	NA	21.5	24.2	(Song and Thornalley, 2007)
		10	Floret (5 cultivars)	1:3	40-58	38-51	NA	39-54	(Volden et al., 2009b)
Green Cauliflower	NA	10-15	NA	NA	58.0	58.3	NC	57.6	(Cieslik et al., 2007)
White Cauliflower					77.9	53.9	NC	64.8	
	Boiling water	10	Floret + stem 2.5 cm	1:5	24.1	78.1	NA	62.5	(Pellegrini et al., 2010)
		9	Floret (initially frozen)		72.8	55.1	NA	61.6	
Curly kale	NA	10-15	NA	NA	30.1	14.9	24.7	27.7	(Cieslik et al., 2007)
Portuguese kale	Boiling water	5	< 0.5 cm	1:5	41.7	31.4	NA	39.5	(Rosa and Heaney, 1993)
Portuguese *B. napus*		10	30-40 cm^2		28.8	31.6	NA	29.5	
Turnips greens		15	NA	1:10	39.5	39.6	0.0	35.9	(Francisco et al., 2010)
	High pressure	5			39.2	38.9	2.4	35.7	
Turnips tops	Boiling water	15			36.0	47.5	22.3	35.5	

[1] = Each study observes two or more GSs. Calculation on totals of aliphatic, indole, and aromatic GSs is based on published mean value of individual GSs belong to the group. Total aromatic is only from gluconasturtiin. These calculation techniques are applied for all processing methods

NA = not available

NC = not calculated; the raw or boiled or both treated *Brassicas* contains traces of GSs

UP = boiling increased gluconasturtiin from traces into certain levels

Steaming

Steaming is applied by exposing *Brassica* vegetable to saturated steam. Steam is commonly produced by vaporizing water under boiling condition and the vegetable is stored in a mesh compartment to prevent from direct contact of boiling water. Heat is transferred mainly by condensation of steam at the vegetable surface and by convection. Since there is no direct contact between the vegetable tissue and a large pool of water, steaming is expected to result in less leaching compared to boiling. The heating rate of the vegetable tissue is generally lower compared to boiling. This leads to lower rates of cell lysis and myrosinase inactivation compared to boiling. In reported studies, in contrast to boiling, steaming either slightly decreased or increased the total or major GS contents of *Brassica* vegetables (Table 2.3). Conaway et al. (2000), Vallejo et al. (2002), and Rungapamestry et al. (2006) have reported no significant effect of steaming on total GS content in cabbage and broccoli with respect to the raw ones. Song and Thornalley (2007) also reported no significant losses of total GSs in broccoli, green cabbage, cauliflower, and Brussels sprouts over 20 min of steaming.

Total GS content in cauliflower was reported to increase by more than two-fold after steaming (D'Antuono et al., 2007). Accordingly, Gliszczyñska-šwiglo et al. (2006) and Miglio et al. (2008) found steaming increased total GS content in broccoli by about 20–30%. The increase of the GS extractability in the analytical methods is apparently higher than the rate of GS thermal degradation upon steaming.

As steaming proceeds myrosinase will finally be inactivated, which will lead to a stable GS content. Rungapamestry et al. (2006) studied time-course effect of steaming of cabbage on myrosinase activity. The authors observed that myrosinase activity remained stable after steaming for up to 2 min but, this showed a loss by 90.4% after 7 min.

In general, it can be concluded that the rates of cell lysis, diffusion, leaching, enzymatic hydrolysis, and thermal degradation are relatively lower during steaming than boiling, which lead to a lower loss of GS content. Moreover, steaming seems to increase the extractability of the GSs.

Table 2.3. Effect of steaming on glucosinolate retention

Brassica	Temperature (°C)	Time (min)	Size	*Brassica* Weight (g)	Water volume (ml)	GS Retention (%)				References
						Aliphatic	Indole	Aromatic	Total	
Broccoli	100 (oven)	13	Floret + stem 2.5 cm	9 specimens	NA	133.7	143.0	NA	138.9	(Pellegrini et al., 2010)
		12	Floret + stem (initially frozen)			81.4	68.2		73.6	
	Boiling water	15	Floret + stem 2.5 cm			124.3	147.0		137.0	
		14	Floret + stem (initially frozen)			92.8	85.7		88.6	
	NA	10	Floret	300		107.2	143.2	140.0	117.1	(Gliszczyñska-šwiglo et al., 2006)
	NA	15	Floret 1.5 inch	200		101.3	NA	NA	76.9	(Conaway et al., 2000)
	Oven	NA	Floret + stem 25 mm	9 specimens		89.5	131.9		130.8	(Miglio et al., 2008)
	Boiling water	3.5	Floret 3 cm dim + stem 1 cm	150	150	111.4	104.5	UP	107.1	(Vallejo et al., 2002)
	~20 - ~100	2-30	Floret 2-3 cm dim + stem 2.5 cm	300	NA	91.5-136.5	44.8-135.1	NA	78.9-131.8	(Verkerk et al., 2010)
	Boiling water	2 or 5	Floret (2 cultivars)	150	150	93.9-106.9	94.6-119.3		96.8-109.6	(Jones et al., 2010)
		5	Piece	200	200	93.3	63.2		84.5	(Yuan et al., 2009)
		5-20	Floret	20-30	500	NA	NA		NS loss	(Song and Thornalley, 2007)
Brussels sprout	100 (oven)	17	Whole	9 specimens	NA	93.9	151.5		125.1	(Pellegrini et al., 2010)
		12	Whole (initially frozen)			83.6	96.8		89.7	
	Boiling water	18	Whole			81.2	117.2		100.7	
		10	Whole (initially frozen)			88.9	82.6		86.0	
		5-20	Whole	20-30	500	NA	NA		NS loss	(Song and Thornalley, 2007)

Table 2.3. Effect of steaming on glucosinolate retention (Continued)

Brassica	Temperature (°C)	Time (min)	Size	*Brassica* Weight (g)	Water volume (ml)	GS Retention (%)				References
						Aliphatic	Indole	Aromatic	Total	
Cabbage		5-20	Whole or largely cut leaves							
Red cabbage		10	1 x 1 cm	300	300	78.1	75.1	91.3	81.2	(Volden et al., 2008a)
Cauliflower		10	Floret (5 cultivars)	300	400	78-84	77-82	NA	78-82	(Volden et al., 2009b)
		5-20	Floret	20-30	500	NA	NA		NS loss	(Song and Thornalley, 2007)
White cauliflower	100 (oven)	13	Floret + stem 2.5 cm	9 specimens	NA	68.7	137.9		118.0	(Pellegrini et al., 2010)
		12	Floret (initially frozen)			106.8	91.7		97.3	
	Boiling water	11	Floret + stem 2.5 cm			50.4	104.3		88.8	
		10	Floret (initially frozen)			89.3	102.3		97.5	
Turnip greens		15	NA	150	1500	86.3	98.8	97.6	90.8	(Francisco et al., 2010)
Turnip tops						75.5	78.3	72.7	78.0	

NA = not available

NS = not significant

UP = steaming increased gluconasturtiin from traces into certain levels

Blanching

Blanching applies similar treatment as boiling or steaming, but can differ in vegetable/water ratio, processing time and temperature. Vegetable is either steamed or submersed into hot or boiling water for few minutes, subsequently followed by a fast cooling. Blanching is considered as a pre-treatment for industrial processing (Fellows, 2009). It was applied on *Brassica* vegetables prior to pickling (Suzuki et al., 2006), freezing, and frozen storage (Rodrigues and Rosa, 1999; Rungapamestry et al., 2008) to reduce further degradation of GSs during the core processing. Blanching of *Brassica* vegetable aims to inactivate myrosinase and consequently, can inhibit the GS enzymatic hydrolysis. Depending on the blanching conditions, the type of GS, and the type of *Brassica* vegetable, lower effects of blanching than boiling are expected on the other mechanisms involved to change GS content, including cell lysis, diffusion, leaching, and thermal breakdown. On the other hand, blanching in water using high water/vegetable ratio will be favouring leaching upon cell lysis and diffusion.

Blanching was reported to reduce total GS content variably (Table 2.4), ranging from about 30% through 52% in cauliflowers (Volden et al., 2009a; Volden et al., 2009b), 30% in broccoli, 2.7% and 13% in green and white cauliflowers, respectively (Cieslik et al., 2007), and 1% through 29% in Brussels sprouts (Goodrich et al., 1989; Wathelet et al., 1996; Cieslik et al., 2007). Volden et al. (2008a) reported a great loss at about 64% of total GSs in red cabbage due to blanching, which is a higher amount than due to boiling in the same study. This could be due to more extensive leaching as the ratio of water to red cabbage for blanching is ten times higher than for boiling, although the blanching time is one-third shorter than boiling time. Wennberg et al. (2006) and Goodrich et al. (1989) accordingly found great GS losses of white cabbage and broccoli, respectively, after blanching. Similar to boiling the main parts of the GS losses in cauliflower (Volden et al., 2009b) and red cabbage (Volden et al., 2008a) were recovered in blanching water.

The differences in type of *Brassica* vegetable and blanching technique could influence the behaviour of GSs over blanching. Goodrich et al. (1989) have compared total GS contents in broccoli and Brussels sprouts after hot water and steam blanching techniques. The authors reported no significant losses of total GS contents in Brussels sprouts after hot water and steam blanching, i.e., by 7% and 22% of initial levels, respectively. On the contrary, these techniques reduced total GS contents in broccoli significantly by 83% and 40%, respectively.

Table 2.4. Effect of blanching[2] on glucosinolate retention

Brassica	Temperature (°C)	Time (min)	Size	*Brassica* weight (g)	Water volume (ml)	GS Retention (%) Aliphatic	Indole	Aromatic	Total	References
Broccoli	80	3	NA	NA	NA	64.7	97.2	NC	69.9	(Cieslik et al., 2007)
	99	4		500		22.4	11.5	NA	17.0	(Goodrich et al., 1989)
	Steam: 99-102	5.5				72.2	47.2		60.1	
Brussels sprouts	80	3		NA		75.5	70.8	79.5	71.9	(Cieslik et al., 2007)
	99	4		500		105.6	88.3	NA	93.2	(Goodrich et al., 1989)
	Steam: 99-102	5.5				74.3	79.4		78.0	
	90 or 95; or steam: 105	2; 5; or 10	32-37 mm dim	NA		82.8-98.9	NA		82.6-99.0	(Wathelet et al., 1996)
Red cabbage	94-96	3	1 x 1 cm	300	3000	34.5	33.5	39.1	35.8	(Volden et al., 2008a)
Cabbage	Boiling water	5	1.5 mm (2 cultivars)	2000	2000	25.1 & 60.6	27.2 & 37.0	NA	25.7 & 49.6	(Wennberg et al., 2006)
Cauliflower	96-98	3	Floret (5 cultivars)	1000	10,000	62.5-75.9	47.8-79.7		58.6-76.3	(Volden et al., 2009a)
				300	3000	56-69	43-70		48-70	(Volden et al., 2009b)
Green Cauliflower	80	3	NA	NA	NA	89.4	119.7	100.0	97.4	(Cieslik et al., 2007)
White Cauliflower						97.7	78.6	NC	87.3	
Curly kale						80.0	74.7	33.3	78.8	

[2] = water blanching; otherwise is specified

NA = not available

NC = not calculated; the raw or blanched or both treated *Brassicas* contains traces of GSs

Table 2.5. Effect of microwave processing on glucosinolate retention

Brassica	Power (W)	Time (min)	Size	*Brassica* weight (g)	Water Addition (ml)	GS Retention (%)				References
						Aliphatic	Indole	Aromatic	Total	
Broccoli	300	30	Floret + stem 2.5 cm	10 specimens	-	97.7	110.4	NA	102.2	(Pellegrini et al., 2010)
		13	Floret + stem (initially frozen)		-	70.8	75.0		73.4	
	500; 700; or 1000	2.5 or 5	Floret 3 cm dim. + stem 1 cm	150	100 or 150	77.4-110.5	57.6-93.8	62.4-87.1	64.3-97.7	(López-Berenguer et al., 2007)
	1100	2 or 5	Floret (2 cultivars)		150	83.6-100.0	86.5-118.7	NA	83.9-104.1	(Jones et al., 2010)
	1000	5	Floret 3 cm dim. + stem 1 cm		NA	15.9	27.4	NC	25.5	(Vallejo et al., 2002)
			Piece	200	10	39.7	46.9	NA	41.8	(Yuan et al., 2009)
	900	0.5-3	Floret	20-30	10% of solid	NA	NA		NS loss	(Song and Thornalley, 2007)
Brussels sprout	300	18	Whole (initially frozen)	10 specimens	-	86.7	148.6		120.3	(Pellegrini et al., 2010)
		6	Whole		-	64.7	99.8		80.9	
	900	0.5-3		20-30	10% of solid	NA	NA		NS loss	(Song and Thornalley, 2007)
Cabbage			Whole or largely cut leaves		10% of solid					
Red cabbage	180; 540; or 900	36 s-24 min	1 cm	300	-	77.5-189.6	92.9-175.8		88.0-178.0	(Verkerk and Dekker, 2004)
Cauliflower	900	0.5-3	Floret	20-30	10% of solid	NA	NA		NA loss	(Song and Thornalley, 2007)
White cauliflower	300	30	Floret + stem 2.5 cm	10 specimens	-	49.8	81.5		72.4	(Pellegrini et al., 2010)
		20	Floret (initially frozen)		-	107.1	117.6		113.8	

NC = not calculated; the raw or microwaved or both treated *Brassicas* contains traces of GSs

NA = not available

NS = not significant

To conclude, cell lysis, diffusion, and leaching during blanching generally lead to substantial loss of GS content. However, inconsistent results are sometimes found which might be due to specific blanching conditions employed and changes in the extraction efficiency of GSs due to heating.

Microwave processing

Processing by using microwave applies a different heating mechanism from other methods employing heat. Microwaves permeate into the food and heat generated within the food is transferred throughout the food by conduction. Food is heated by rotation of the dipolar water molecules and translation of the ionic components of food during the absorption of microwave energy. The main intrinsic factors involved, therefore, are the water content and the dissolved ion content of the food (Ohlsson and Bengtsson, 2001).

Several mechanisms, particularly cell lysis, diffusion, and myrosinase inactivation, take place during microwave processing determining the fate of GS content. Degree of changes of GS content is strongly affected by processing time and power output. Longer treatment duration will increase plant cell lysis and thermal degradation. Meanwhile, the activity of myrosinase will increase at moderate heat at temperatures up to about 60 °C and inactivation will occur rapidly at higher temperatures (Verkerk and Dekker, 2004). Leaching is only expected when a considerable amount of water is added to the vegetable prior to microwave processing (Vallejo et al., 2002; López-Berenguer et al., 2007).

Microwave processing can retain the total GS contents (Table 2.5) in cabbage, broccoli, Brussels sprouts and cauliflower (Fuller et al., 2007; Song and Thornalley, 2007). Verkerk and Dekker (2004) studied the effect of microwave on the GSs in red cabbage by combining each of three output powers (i.e., 180, 540, and 900 W) and each of five different processing times (over 24 min). Despite of few losses of total GSs, the authors found that these combinations increased total GS content. These were expected due to higher chemical extractability of the plant tissue over heating. Meanwhile, Rungapamestry et al. (2006) studied the time-course effects of processing by microwave at 750 W and six time intervals over 7 min on cabbage. The authors found the loss of total GS content in cabbages by 17.3% after microwave processing for 7 min. Accordingly, López-Berenguer et al. (2007) reported general decrease of total GSs in broccoli which was added with 150 mL of water after microwave processing for 5 min at different power levels.

The activity of myrosinase can diminish with increasing energy inputs (Verkerk and Dekker, 2004). Microwave processing at 900 W and the highest energy inputs almost completely reduced hydrolytic capacity of myrosinase in cabbage. The milder microwave-treated cabbage at 540 W resulted in a reasonable amount of myrosinase residual activity capable of converting the exogenous sinigrin even at higher energy inputs. Furthermore, cabbage treated at the lowest microwave-power retained the highest myrosinase activities (Verkerk and Dekker, 2004). Accordingly, Rungapamestry et al. (2006) reported that myrosinase activity in cabbage initially decreased by 27.4% after 45 sec of microwave processing at 750 W and further decreased by 96.7% after 2 min.

Leaching of GSs was expected when considerable amount of water is added prior to microwave processing (Vallejo et al., 2002; López-Berenguer et al., 2007; Jones et al., 2010). Vallejo et al. (2002) reported that very intensive microwave processing for 5 min at 1000 W caused a great loss of total GS content of broccoli florets by about 74% but, the recovery of total GSs was only about 1% in water. This was expected due to loss of cooking water containing leached GSs (Vallejo et al., 2002); unfortunately, the amount of evaporated water was not reported. When a considerable amount of water is lost from the vegetable tissue, the temperature by the microwaves can easily increase to values substantially above 100 °C inducing rapid thermal breakdown. A similar microwave processing condition on broccoli, however, reduced about 18% of total GS content and parts of this loss were recovered in the cooking water (López-Berenguer et al., 2007). Although the amount of additional water was considerably small, the great loss of total GSs in broccoli by about 60% after microwave processing for 5 min was also reported when high power was employed at 1000 W (Yuan et al., 2009).

So, the mechanisms of cell lysis, diffusion, thermal degradation, and leaching (when additional water is added) are involved in reducing the GS content during microwave processing. But, the conflicting results might be due to specific microwave processing conditions applied and the effect of heating on the extraction efficiency of GSs.

Stir-frying

Stir-frying is a quick vegetable processing method using small amount of preheated cooking oil. Heat is transferred mostly by conduction performed from the hot surface of the pan or wok through a thin layer of oil. Consequently, the surface temperature of vegetable rises rapidly and a proportion of water is vaporised (Fellows, 2009). Stir-frying applies high temperature of cooking oil and shorter time than many other processing methods. The main part of the vegetable tissue will not exceed 100 °C for the short processing times usually applied, since the tissue will still contain most of its water content. Small amount of water might be added during stir-frying, depending on the local custom, type of *Brassica* vegetable, and the expected product. Low degrees of cell lysis and diffusion, leaching, thermal degradation, and enzymatic hydrolysis can be expected to occur during stir-frying of *Brassica* vegetable.

Stir-frying could retain the total GSs and most of individual GS content in green cabbage, broccoli, Brussels sprouts and cauliflower (Song and Thornalley, 2007; Rungapamestry et al., 2008). Rungapamestry et al. (2008), furthermore, reported that when the temperature of broccoli during stir-frying was maintained at about 80 °C myrosinase activity was reduced by 83%. The authors found no significant effect of myrosinase activity on the GS loss. However, Yuan et al. (2009) observed that stir-frying of broccoli reduced considerable amount of the aliphatic and indole GSs by about 55% and 67%, respectively, possibly due to extensive time-temperature employed (Table 2.6).

During stir-frying, thermal degradation mechanism might involve predominantly than leaching to degrade GS content. Yuan et al. (2009) compared two stir-frying techniques on broccoli, namely the with and without external water, and found no significant difference of GS losses between these techniques. When deep-frying was applied, total GSs in broccoli reduced considerably by 84% apparently due to the intense thermal degradation (Miglio et al., 2008). In addition, blanch-freezing treatment prior to stir-frying of broccoli was reported to not significantly change the total GS content particularly for aliphatic and aromatic GSs (Rungapamestry et al., 2008).

The types of cooking oil used for stir-frying might also influence on the fate of GS content. Stir-frying using refined olive and sunflower oils reduced total GS content in broccoli florets significantly by 49% and 37%, respectively, with respect to the uncooked controls. Meanwhile, stir-frying using extra virgin olive, soybean, peanut, or safflower oils relatively gave no significant effect on the GS content (Moreno et al., 2007). The authors, however, did not observe relationship between the cooking temperature or the lipid composition of the oils and the exerted effect on the GS content**.**

To conclude, limited or no leaching, enzymatic hydrolysis, and thermal degradation occur during short stir-frying slightly reduce the GS content. However, inconsistent results are sometimes found which might be due to specific stir-frying conditions applied and the effect of heating on the extraction efficiency of the GSs.

Table 2.6. Effect of stir-frying on glucosinolate retention

Brassica	Oil temperature (°C)	Time (min)	Size	*Brassica* weight (g)	Oil volume (ml)	Water addition (ml)	GS Retention (%)				References
							Aliphatic	Indole	Aromatic	Total	
Broccoli	Hot olive oil Broccoli temp.: 80	2 + 2*	Floret 40 mm length	110	5	7	99.6	98.5	96	98.7	(Rungapamestry et al., 2008)
	125-140	3.5	Floret 3 cm	150	40	-	NA	NA	NA	51-~100	(Moreno et al., 2007)
	130-140	5	Piece	200	10	-	45.0	36.2		41.6	(Yuan et al., 2009)
		2 + 3*				50	42.4	37.8		43.4	
	Pre-heated to 200; kept at 110-120	3 & 5	Strips 1 cm	20-30	15% w/w	NA	NA	NA		NS loss	(Song and Thornalley, 2007)
Brussels sprouts											
Cabbage											
Cauliflower											
Deep-frying											
Broccoli	170 °C	NA	Floret + stem 25 mm	10 specimens	2200	-	31.6	15.5		16.0	(Miglio et al., 2008)

* = additional cooking time after the addition of water

NA = not available

NS = not significant

Fermentation

Fermentation is one among the old processing methods and have formed a traditional part of the diet in many countries (Fellows, 2009). The fermented *Brassica* vegetable commonly studied, particularly in European region, is sauerkraut (de Vos and Blijleven, 1988).

Fermentation of *Brassica* vegetable involves the growth and metabolism activity of lactic acid bacteria, either spontaneously or by starter-induced, and sodium chloride to produce fermented products (Tolonen et al., 2002). Mechanisms affecting GS content differ from processing by applying heat; however, to our knowledge these are scarcely studied. Bacteria and sodium chloride might take significant role to change the GSs during fermentation (Tolonen et al., 2002; Suzuki et al., 2006). Suzuki et al. (2006) examined in vitro myrosinase activity against sinigrin at various levels of NaCl and pH. The authors observed no myrosinase activity in the presence of 500 mM NaCl and below pH 5.5.

Fermentation was reported to reduce total GS content substantially. No GS content was observed in fermented cabbage and stored sauerkraut (Daxenbichler et al., 1980; Ciska and Pathak, 2004), irrespective of cabbage cultivation season, fermentation type, and salt concentration (Martinez-Villaluenga et al., 2009). Tolonen et al. (2002) observed 4-methoxy-glucobrassicin in the final fermented cabbage in small quantities. Meanwhile, Suzuki et al. (2006) reported that the GSs in *nozawana-zuke*, a fermented product of *Brassica rapa* L., were glucobrassicin and gluconasturtiin as the major GSs, and gluconapin, 4-hydroxy-glucobrassicin, glucoberteroin, and 4-methoxy-glucobrassicin in small quantities relative to the fresh *nozawana*.

Ciska and Pathak (2004) detected the breakdown products of the GSs in sauerkraut, included ITCs and cyanides from aliphatic GSs, indole-3-carbinol, indole-3-acetonitrile, and ascorbigen from glucobrassicin, and 2-phenylethyl ITC from gluconasturtiin. Meanwhile, Daxenbichler et al. (1980) observed thiocyanate ion from glucobrassicin and 1-cyano-3-methyl sulfinylpropane from glucoiberin throughout cabbage fermentation. Tolonen et al. (2002) also reported allyl ITC, allyl cyanide, methyl ITC, indole-3-carbinol, goitrin, sulforaphane and sulforaphane nitrile, 3-methylsulfinylpropyl ITC, and buten-4-ITC in the fermented cabbage. Ascorbigen was reported as a major derivative of GS degradation products identified in fermented cabbage (Ciska and Pathak, 2004; Martinez-Villaluenga et al., 2009) but, this was not detected in *nozawana-zuke* (Suzuki et al., 2006). Furthermore, various tendencies and rate of changes in the content of GS degradation products were found during storage of fermented cabbage (Ciska and Pathak, 2004). The authors suspected that the content of degradation products in fermented cabbage does not depend only on the content of native GSs in raw cabbage but, it also substantially depends on physicochemical properties such as volatility, stability, and reactivity in an acidic environment, as well as microbiological stability.

Suzuki et al. (2006) used watercress as a model to examine the behaviour of individual GS during fermentation. The authors reported that the GS content decreased after seven days of fermentation but, the ratio of indole GSs to total GSs was much higher than before fermentation. It was proposed that a stress response under salting treatment or compression and/or myrosinase resistance are the possible factors. In addition, they found that a shorter maturation period retained more GS content. Leaching, meanwhile, hardly involve in the fermentation as most GSs were retained in the tissues and no GSs were detected in the brine (Suzuki et al., 2006).

To summarize, fermentation can reduce the amount of GSs in *Brassica* vegetables considerably. Bioconversions are the most likely cause of this loss; however, further studies are needed to identify and explain the working mechanisms underlying the GS degradation.

Other processing methods

The effects of freezing, drying, and pressure/temperature processing have also been studied on the GS content in *Brassica* vegetables (Rungapamestry et al., 2008; Van Eylen et al., 2009; Mrkic et al., 2010). Freezing applies sub-zero temperature treatment to below the freezing point and changes a proportion of water into ice crystals. Drying applies moisture content removal commonly by circulation of hot dried air on the vegetable surface. Meanwhile, high hydrostatic pressure in combination with mild temperatures is an alternative to thermal processing mainly to inactivate microorganisms yet retain the beneficial compounds.

The GS content of *Brassica* vegetables can be retained during freezing and frozen storage. Rungapamestry et al. (2008) reported that freezing the blanched broccoli at -18 °C within 20 min by using air blast freezer did not substantially change the total GS content. The contents of glucoiberin, glucoraphanin, and gluconasturtiin were retained and gluconapoleiferin content was increased by 50% but, glucobrassicin and 4-methoxy-glucobrassicin contents were reduced significantly. Blanch-freezing, furthermore, also reduced myrosinase activity in broccoli by 93%. In accordance, Rodrigues and Rosa (1999) reported that freezing the blanched broccoli at -20 °C retained the total GS content in the principal inflorescences but, significantly decreased the total GS content in the secondary inflorescences. The authors suspected that the loose structure of the broccoli stalk and flower head are very susceptible to the leaching effects during prior blanching.

During storage of blanch-frozen broccoli at -20 °C for 90 days, the GS content and myrosinase activity were generally unaltered, except for neoglucobrassicin (Rungapamestry et al., 2008). Accordingly, Volden et al. (2009a) found no significant effects of frozen-storage of cauliflower at -24 °C for 12 months on the total GS content. Significant losses of GS content were found on specific cultivars after three months of storage. The individual GSs, moreover, were not considerably altered throughout the long-term frozen-storage. Cieslik et al. (2007) however, reported that prolonged freezing of blanched *Brassica* vegetables at -22 °C for 48 hours did not produce any consistent changes in total GS content. Losses of total GS content relative to the blanched vegetables were 50.7% in frozen Brussels sprouts and 4.5% in frozen curly kale. In contrast, the total GS contents in frozen green cauliflower and broccoli increased by 20.9% and 28.5%, respectively. Meanwhile, Song and Thornalley (2007) reported that storage of broccoli, Brussels sprouts, cauliflower, and green cabbage at -85 °C for two months without prior blanching caused significant loss of GSs. The authors suspected that this is due to freeze-thaw fracture of plant cells and accessibility of myrosinase to GSs during thawing. Freezing ruptures plant cells and softens vegetables because of water crystallization in extra-cellular and intra-cellular spaces within the vegetable matrix.

The effects of drying conditions, namely temperature (at 50 through 100 °C) and drying air velocity (at 1.2 through 2.25 ms^{-1}) on the content of indole GSs in broccoli were examined (Mrkic et al., 2010). The authors found that different drying conditions affected individual GS differently. The remaining individual GSs of the dried relative to the blanched broccoli were in the range of about 32–90% for 4-hydroxy-glucobrassicin, 65–92% for glucobrassicin, 29–90% for 4-methoxy-glucobrassicin, and 36–92% for neoglucobrassicin. Moreover, at the same air velocity the technique of drying by decreasing temperature did not affect to the better GS content than the effect of drying at constant temperature.

A high pressure/temperature treatment on *Brassica* vegetable offers an advantage over other conventional processing methods. This processing can bring an active form of myrosinase into contact with the GSs and consequently, improve the health beneficial compound production (Van Eylen et al., 2009). Van Eylen et al. (2009) reported that GS hydrolysis could be induced during mild pressure processing of broccoli. About 20% loss of GSs was observed after 35 min of treatment at 20 °C and elevated pressure (200–300 MPa). Meanwhile, the GS degradation was found after 15 min of treatment at 40 °C and 100–500 MPa and the highest lost, i.e., 63% of GSs, was observed at 300 MPa. Furthermore, the hydrolysis products of the aliphatic GSs might disappear from the broccoli after treatment, while the hydrolysis products of the glucobrassicin were formed during treatment. The authors indicated that by varying the process parameters, e.g., time, pressure, and temperature, the extent of GS hydrolysis can be altered, which will lead to different amounts of health beneficial products. Finally, besides high pressure processing, other novel food processing technologies are emerging in food manufacturing such as pulsed electric field, cold plasma and advanced heating technologies including microwaves, ohmic- and radio frequency heating. Possible effects of these new technologies on GSs in *Brassica* vegetables and the potential health benefits of derived products should be studied in future.

Cultural differences in cooking practices of *Brassica* vegetables

Studies on processing effect of *Brassica* vegetables on the GS behaviour to our knowledge are mainly carried out in the Western perspective. Nevertheless, *Brassica* vegetables can be prepared and cooked by a range of preparation methods, involving a combination of techniques and ingredients. Preparation methods could reflect the diversity of food culture, which refers to the ways in which humans use food (Kittler and Sucher, 2008). It is neither the intention of this section to differentiate food culture between regions nor to emphasize food identity but, to bring the cultural perspective on various food preparation and cooking methods in relation to the effect on the content of GSs.

Van Esterik (2008), for example, had thoroughly described the variety of food culture in Southeast Asia. In general, the meals require complexity of processing and preparation but, cooking times are short. The basic attributes for the meals are the harmony of taste and texture and the balance of hot/spicy, sour, salty, and sweet. Food processing takes place in rural and urban households as well as in small and large-scale industries. Street vendors and mobile food, for examples, are common in urban areas, providing a wide range of dishes to go with rice, to eat-on-site or take-it-away, from early morning to late-night. There is an almost-infinite variety of foods available on the streets.

For the context of *Brassica* vegetables, various preparation and cooking methods are commonly employed in Southeast Asian countries. For example, stir-frying, one of the popular processing methods (Van Esterik, 2008), is employed by quickly stir-frying the vegetables over high heat of small amount of oil, either with or without external water. The vegetables are cooked together with spices and could be combined with small amounts of meat or fish. For fermentation of *Brassica* vegetables, *dhamuoi*, *dakguadong*, *burong mustala* (Lee, 1997), and *sayur asin* (Puspito and Fleet, 1985) are examples of popular fermented products in some Southeast Asian countries.

Furthermore, for boiling and steaming, various preparation techniques can be employed ranging from mild to extensive (heat) treatment, depending on the types of *Brassica* vegetables and the corresponding products. To illustrate, in Indonesia, preparation of white cabbage ranges from short boiling or steaming to few hours steaming for producing for examples parts of the ingredients of *pecel* and *siomay* dishes, meanwhile choysum (*Brassica rapa* var *parachinensis*) can be prepared by quickly immersing in, or poured by hot water (or be boiled for a few longer period) to be added in the meatball or noodle soups. In addition, in Asian cooking practices there is a wide variability of other ingredients included in the preparation. Moreover, some soups containing *Brassica* vegetables, for example, are found in soft-textured form due to prevalent application of extended boiling. Particularly in food service establishments, e.g., street food vendors or catering service, vegetable based dishes are sometimes reheated to maintain the consumers' appetite. They consider the efficiency and convenience by having the dish prepared once a day for cater the whole day consumers, while at the same time the textural quality of the corresponding product is compromised.

The variability of preparation methods above indicates the diversity of potential impacts to the GS content in *Brassica* vegetables. Some of the methods may reduce the GS content significantly while others may retain most of their content. To our knowledge, these specific preparation methods are scarcely studied. Therefore, a study on the effects of processing of *Brassica* vegetables commonly practiced in Southeast Asian countries, for example, will be valuable for promoting health in a developing society.

Conclusions and future studies

Processing aims to improve beneficial properties of *Brassica* vegetables, such as improved palatability and bioavailability of nutrients, and shelf life extension. Processing, however, changes the content of health promoting GSs diversely, depending on the processing method and conditions, the type of *Brassica* vegetable, and the type of GSs. A mechanism approach underlying each processing method can explain the behaviour of GS content. Boiling and blanching can reduce the GS content considerably due to mechanisms of cell lysis, diffusion, thermal degradation, and leaching. Fermentation also reduces GS content of *Brassica* vegetables; however, more mechanistic-based processing studies should be further performed to reveal breakdown mechanisms of GS content thoroughly. Steaming and microwave processing relatively retain or even increase total accessible GS content because of low impact on mechanisms of cell lysis, diffusion, thermal degradation, and leaching but can increase the extractability of GSs in plant matrix. Stir-frying may slightly reduce GS content in a lower level than boiling and blanching. Meanwhile, changes of individual GS are more intricate to be explained, involving individual GS properties in each type of *Brassica* vegetable and different responses to each processing method and condition.

Further studies on the effect of processing of *Brassica* vegetables by using an approach of mechanisms underlying each processing type will contribute to understand thoroughly the behaviour of GS content under processed. Studies on various preparation methods from other regions and culture will also enrich the understanding of the GS behaviour in specific conditions. In addition, optimizing GS content in *Brassica* vegetables by using a kinetic model describing the main mechanisms involved will play a vital role for fundamental consideration of further epidemiological or product/process design studies.

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Chapter 3

Practices and health perception of preparation of *Brassica* vegetables: translating survey data to technological and nutritional implications

Probo Y. Nugrahedi, Inneke Hantoro, Ruud Verkerk, Matthijs Dekker, and Bea Steenbekkers

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Abstract

Food preparation practices are known to have large nutritional implications on the final product. This paper describes survey data on preparation practices of *Brassica* vegetables and the translation of these data into technological and nutritional implications using knowledge on the mechanisms of changes in the content of phytochemicals. The survey on preparation practices was performed with food service establishments (*n*=123) and households (*n*=477) in Semarang, Indonesia, and assessed the food handlers' perception of the health benefits of these vegetables. Boiling and stir-frying are the most frequently applied techniques to prepare *Brassicas*. The respondents perceive that steaming, boiling, and stir-frying result in vegetables with a high health benefit. White cabbage and choysum are the most frequently prepared *Brassicas*. However, broccoli is perceived as the healthiest. The consequences of the various applied preparation techniques on the content of alleged health promoting phytochemicals (glucosinolates) in dishes containing *Brassica* vegetables are discussed.

Keywords *Brassica* vegetables, preparation practices, food service establishment, household

Introduction

Previous studies reported the substantial contribution of fruit and vegetable consumption toward promoting health (Bazzano et al., 2002; Liu, 2003; Dauchet et al., 2006). In particular, intake of *Brassica* vegetables, such as broccoli, cabbages, Brussels sprouts, and cauliflower, is inversely associated with the risk of certain types of cancer and cardiovascular diseases (Higdon et al., 2007; Herr and Büchler, 2010; Soengas et al., 2011).

Vegetables are usually prepared into various dishes and are consumed on a daily basis. Food preparation techniques, particularly that involving heat aim to inactivate pathogens, to increase sensorial liking and digestibility, and other beneficial changes, but it can also lead to negative impacts, such as loss of certain (micro)nutrients and phytochemicals, formation of toxic compounds or of compounds with negative effects on sensorial perception (van Boekel et al., 2010). Previous studies reported that different preparation techniques performed on *Brassica* vegetables could diversely affect the sensorial acceptance (Poelman and Delahunty, 2011; Poelman et al., 2013) and unfortunately, decrease the content of health promoting compounds, such as glucosinolates, vitamin C, and polyphenols (Miglio et al., 2008; Pellegrini et al., 2010; Bongoni et al., 2013; Bongoni et al., 2014).

Preparation of food is one of the activities individuals or groups perform regarding food. Previous studies described that various factors influence the association between food preparation and consumers' health (Gittelsohn et al., 1998; West et al., 1999; Larson et al., 2006). Others reported the relationship between food preparation behaviour of the food handlers and food safety, particularly related to food borne illnesses (Woodburn and Raab, 1997; Green et al., 2005; Green and Selman, 2005). However, to our knowledge the documentation on practices of vegetable preparation and the food handlers' perception on health is still scarce.

This paper describes *Brassica* vegetable preparation techniques performed by food handlers in food service establishments and households. The respondents' health perception of both the vegetables and the preparation techniques is also presented. Since the popularity of eating out of home is becoming more widespread in Asian countries, the role of food service establishments in providing daily meals to people is inevitably important. Nevertheless, the family member still plays a vital role in providing the meals at home. *Brassica* vegetables were chosen in this study because these are commonly prepared by many food service establishments and families in Asia. Furthermore, these vegetables share common health promoting compounds, namely glucosinolates, found almost exclusively in the *Brassicaceae* family. The information on preparation practices of *Brassica* vegetables gathered in this survey is integrated with knowledge on the mechanisms of changes in the content of glucosinolates (Nugrahedi et al., 2015) for identifying the technological and nutritional implications.

Methods

A questionnaire was designed, consisting of questions on the type of *Brassica* vegetables most frequently prepared, the type of preparation techniques most frequently applied on the *Brassica* vegetables, the name of the dishes prepared from the vegetables, and the perception on the health benefit of the vegetables as well as of the preparation techniques. The survey was conducted in Semarang city, the capital of Central Java province, Indonesia from March 2010 to January 2011. Two groups of respondents were included, namely food handlers in food service establishments and in households. The data collection was divided into two parts. In part I the respondents were given a short questionnaire on *Brassica* vegetable preparation and the respondents' willingness to participate in the second part of survey was asked. In part II more detailed questions were asked. Results from both parts are used in this study.

Since the target population of the survey has no habit of making appointments, respondents were randomly visited in the food service establishments or at home by the interviewer and asked for his/her willingness to be involved in the research. An individual interview was performed by using the questionnaire, where in general the interviewer registered the responses. Some respondents however, preferred to fill in the questionnaire by themselves under supervision of the interviewer. The questionnaire consists of both closed and open questions. For some closed questions an option was given to fill in other answers than the provided ones. For some questions different types of answering categories were used for the food service establishments and the households, i.e., ranking and multiple-choice types, respectively. The data were tabulated and descriptively analysed using Excel office.

In order to assure that respondents know the types of *Brassica* vegetables, the vegetables were described or shown on a picture to the respondent prior to the interview. Furthermore, the respondents were informed that non-*Brassica* vegetables, such as spinach, water spinach, lettuce, papaya leaf, cassava leaf, and celery, are not part of the questionnaire.

Food service establishments

The food service establishments were categorized into three subgroups, namely restaurants, permanent vendors, and non-permanent street vendors. Another type of food service establishment, i.e., catering, was excluded from the sample. A food service establishment is categorized as a **restaurant** when it has a permanent building (sometimes it has a sign 'restaurant' at the building), it has more facilities than other food service establishments and can be a part of a hotel. The dishes are mostly freshly prepared and various dishes are available, although several of these restaurants also serve already prepared dishes. A 'freshly prepared dish' is prepared after being ordered by the customer, while an 'already prepared dish' was prepared earlier and displayed in a way that the customer can see the available dishes and choose what to buy. A **permanent vendor** is located in a permanent building and the dishes served are mostly already prepared, although some of them also serve freshly prepared dishes. The types of dishes are less varied than in a restaurant. A permanent vendor often has better access to water than a non-permanent vendor, and can be a part of a canteen or food-stalls centre. A **non-permanent street vendor** is located in a non-permanent stall nearby the street, mostly tent type. The dishes served are mostly freshly prepared, although some of them also have already prepared ones.

For the food service establishments, part I of the survey was carried out among 40 respondents divided over the three subgroups. Subsequently, part II of the survey was performed involving 123 respondents, consisting of 13, 70, and 40 respondents in the restaurant, permanent vendor, and non-permanent street vendor groups, respectively. All respondents of part I were also involved in part II of the survey. The participating food service establishments are mainly located at or nearby the city centre, campuses, main streets, or dense settlements.

Households

Part I of the survey for the households involved 200 respondents coming from four areas in Semarang city, i.e., *Perumahan Tlogosari*, *Perumahan Ngaliyan*, *Kelurahan Gabahan*, and *Kelurahan Patemon*, in which each area was represented by 50 respondents. The subsequent survey (part II) was also conducted in these areas involving 477 respondents and comprised of 136, 106, 115, and 120 respondents from those areas, respectively. Out of the former 200 respondents, 71 respondents were involved in the subsequent survey.

Types of preparation technique

Various techniques can be performed to prepare *Brassica* vegetables. In each preparation and type of dish there are also various, yet subtle, differences among food handlers in both food service establishments and households. **Preparation** in this study refers to the method or technique applied to process the raw *Brassica* vegetable into a dish. This excludes the preparation of the ingredients prior to cooking, such as washing, grinding, cutting, or chopping. Furthermore, the **dish** itself is defined as the product of vegetables, including *Brassica* (and other ingredients) after preparation. The non-vegetable based products accompanied with a small amount of *Brassica* vegetable to make a complete dish were also included here. Since white cabbage is also eaten raw as part of a dish, this is considered a dish as well. Except for blanching and steaming, the vegetable preparation usually involves adding spices and seasonings, e.g., garlic, onion, shallot, hot chili, salt, sugar, pepper, sauce, and ketchup. The vegetables can also be prepared together with other ingredients such as meat, seafood, and other types of (non-*Brassica*) vegetables.

Boiling is putting the vegetable into either hot or boiling water for some time and it is usually eaten as a soup, including the water. **Stir-frying** is performed as a few minutes frying the vegetable into a small amount of hot cooking oil with regular manually stirring. Depending on the type of dish or vegetable and the amount of moisture released from the vegetable, a small amount of water or thickener (e.g., maize solution) can be added during stir-frying. **Frying** is performed by cooking the vegetable or batter-coated vegetable in a larger amount of hot oil than used for stir-frying. **Steaming** is employed by putting the vegetable in steam from a boiling water source. For the context of the current survey, **blanching** is defined as very light boiling or immersing the vegetable in- or pouring with- boiling or hot water for a short time. The blanching water is usually not used as part of the dish. Other techniques, such as **microwave cooking** and **baking** are relatively uncommon in Indonesia for preparing vegetables. Another type of preparation commonly performed on *Brassica* vegetables is **fermentation**. Fermentation is usually employed by the food service establishments in the production of *sayur asin* made from Indian mustard (*Brassica juncea*) (Puspito and Fleet, 1985). However, this was not included in the survey because the *sayur asin* is commonly further prepared (e.g., by boiling to make soup).

Statistical analysis

A descriptive analysis of the data was performed by using Microsoft Excel to calculate frequency distributions.

Nutritional implication of preparation techniques used

The nutritional implications of the used preparation techniques will depend on the exact conditions applied. Although the survey information does not provide this information in detail, the expected changes in phytochemical (glucosinolate) intake are discussed by considering the general effects of the preparation techniques on various possible mechanisms for loss as described by Nugrahedi et al. (2015), namely cell lysis, leaching, enzymatic inactivation, and thermal degradation.

Results

Food service establishments

Characteristics of respondents

Table 3.1 presents the characteristics of the food service establishment respondents in the study. The majority of the respondents (63%) are the chefs or cooks (including combined chefs or cooks and owners) and 24% are owners of the establishment. Although the owners are usually not the food handlers, they play a significant role regarding the policy and the mode of operation of the food service establishment. In 55% of the food service establishments, the dishes involving *Brassica* vegetables are freshly prepared and in 33% of the food service establishments already prepared dishes are served. The rest of the food service establishments prepare both types of dishes. The majority of the respondents (89%) purchase raw vegetables every day and the rest every two days.

Table 3.1. Characteristics of the food service establishment respondents in the study (n = 123)

	N	(%)
Status		
Owner	30	(24)
Chef or cook	8	(7)
Owner and Chef or cook	69	(56)
Others (e.g., manager, supervisor, and servant)	16	(13)
Gender		
Female	60	(49)
Male	63	(51)

Types of Brassica vegetables used

The respondents were asked to rank seven commonly available types of *Brassica* vegetables according to the frequency of preparation. Figure 3.1 shows the distribution of this ranking and the number of respondents per rank. The decreasing number of respondents implies that not all vegetables are prepared by all food service establishments. White cabbage is the most frequently prepared *Brassica* followed by choysum. These vegetables are ranked as number 1 by 56% and 32% of the total number of respondents, respectively. Beside Chinese cabbage, these vegetables are also dominant at the second rank (23% and 33% respectively, of 78 respondents). Less frequently prepared *Brassicas* are broccoli and cauliflower. Chinese kale and pakchoy are the least frequently prepared *Brassica* vegetables in the food service establishments.

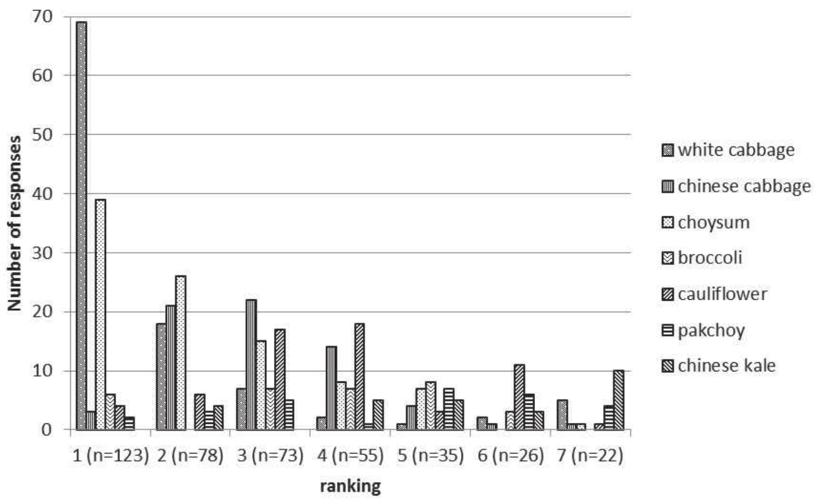

Figure 3.1. Ranking distribution of the frequency of *Brassica* vegetables prepared by food service establishments (rank 1 = the most- to rank 7 = the least frequently prepared; number of respondents for each rank is shown between brackets)

Preparation techniques

The distribution of the ranking on the techniques commonly performed by the food service establishments to prepare *Brassica* vegetables is shown in Figure 3.2. Clearly, boiling is the most dominant technique to prepare *Brassica* vegetables and ranked first by about 58% of the total 123 respondents, followed by stir-frying (about 25%) at the first rank. These are also the most dominant techniques at the second rank, i.e., 31% and 54% of the 74 respondents, respectively. Meanwhile, blanching, steaming, and other techniques (in this case mainly frying) are less frequently employed.

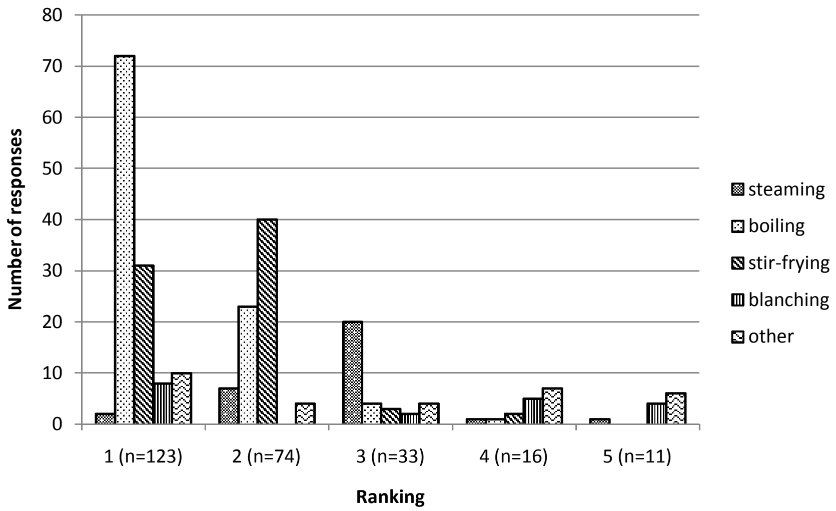

Figure 3.2. Ranking distribution of the frequency of preparation techniques performed by food service establishments (rank 1 = the most- to 5 = the least frequently applied; number of respondents of each ranking is shown between brackets)

Type of prepared dishes

Various dishes containing *Brassica* vegetables are prepared by the food service establishments (Table 3.2). White cabbage followed by choysum are prepared into a larger variety of dishes than other vegetables, particularly to accompany the non-vegetable based dishes, e.g., any type of noodles (*mi*, *kwetiau*, *ifumi*), fried rice (*nasi goreng*) or meatball and dumpling soup (*bakso*, *pangsit*). A lower number of dishes is prepared from Chinese cabbage and cauliflower while Chinese kale, broccoli, and pakchoy are used only in some dishes. These all *Brassica*-containing dishes are prepared with other ingredients and mainly consumed as a side dish for rice.

The dishes are briefly described in the following paragraph, while the details can be found elsewhere (e.g. Brissenden, 2003 and many recipe books). The ***cap cay*** (chop suey) dish is prepared by stir-frying a mixture of various vegetables, including *Brassicas*, and other ingredients, with or without small amount of water added during preparation. Some amount of water can also be added followed by boiling to produce the *cap cay* soup.

Table 3.2. Dish variety and number of respondents from food service establishments (n = 123) that mention the respective *Brassica* vegetable as a commonly used ingredient for the dish and its preparation technique.

Vegetable based dishes	Commonly applied technique	Number of vegetables	White cabbage	Choysum	Chinese cabbage	Broccoli	Cauliflower	Chinese kale	Pakchoy
Cap cay (Chop suey)	Stir-fry/boil	Mix	42	44	37		33	2	
Sup (Soup)	Boil	Single or mix	55	6	10	3	51	1	1
Ca/tumis/oseng	Stir-fry	Single or mix	2	18	34	22	2	18	20
Lalapan	Raw/boil	Single or mix	24			1			
Pecel; *gado-gado*	Boil/blanch	Mix	15	1	1				
Orak arik	Stir-fry	Single or mix	9		1		1		
Sayur asam (Tamarind vegetable soup)	Boil	Mix	3						
Gudangan; *urap*	Boil/blanch	Mix	2						
Non-vegetable based dishes									
Mi, *kwetiau*, & *ifumi* (Noodles)	Boil/stir-fry/blanch	Single or mix	53	77			1		
Bihun (Rice vermicelli)	Stir-fry/boil	Single or mix	11	12					
Bakso & *pangsit* (Meatball & dumpling)	Boil	Single		13					
Bakwan	Fry	Single or mix	9						
Tahu isi	Fry	Mix	5						
Siomay (kind of dim sum)	Boil and steam	Single	4						2
Tahu campur; *tahu gimbal*	Fry	Single	3						
Nasi goreng (fried rice)	Raw	Single	3						

Meanwhile, ***sup*** (soup) is the dish prepared by boiling one type of *Brassica* or a mixture of vegetables and other ingredients in boiling water. ***Sayur asam*** (tamarind vegetable soup) can also be categorized as soup. The ***ca***, ***tumis***, or ***oseng-oseng*** dish is a dish prepared by stir-frying either a single *Brassica* or a mix of vegetables, and other ingredients. Differences in ingredients or spices may determine the variety of the dish names. Nevertheless, many respondents use the terms interchangeably. ***Lalapan*** is a kind of salad, mostly comprised of sliced raw white cabbage and other ingredients, such as tomato, cucumber, and lemon basil, and usually be served with chili sauce. The *lalapan* is a complementary dish, served together with a main dish, e.g., fried chicken, fried fish, fried rice, and satay. ***Pecel*** and ***gado-gado*** contain a mixture of light boiled or blanched vegetables and other ingredients dressed with peanut sauce. The difference between these dishes is the variety of ingredients and sometimes the sauce. ***Gudangan*** or ***urap*** is also a mixture of lightly boiled or blanched vegetables, including white cabbage, dressed or mixed with roasted or stir-fried shredded coconut and a spices mixture. ***Orak-arik*** is a mixture of stir-fried white cabbage and egg. Other vegetables and ingredients can also be included. Furthermore, *Brassica* vegetables are also served as minor part of main dishes, such as any types of noodles, vermicelli, snacks (*bakwan*, *tahu isi*), meatball soup, etc. The vegetables are mostly prepared together with the main ingredients.

Perception on health

Out of 123 respondents, 41% agree with the statement that preparation can reduce the health benefit of the *Brassica* vegetables. Another 26% of the respondents disagree and the rest (33%) does not know. Figure 3.3 presents the frequency distributions of the scores on perceived health benefit of consuming some specific *Brassica* vegetables. Broccoli is perceived as the most health-beneficial *Brassica* (38% gave score 1) followed by choysum (28.5% gave score 1). Respondents perceived pakchoy, Chinese cabbage, and Chinese kale lower in health benefit. *Brassicas* perceived lowest in health benefit are cauliflower and white cabbage.

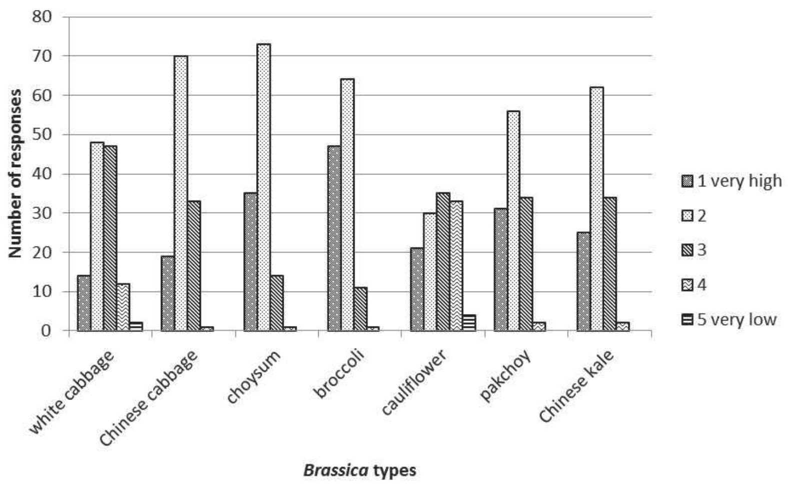

Figure 3.3. Frequency distribution of the scores on perceived health benefit per *Brassica* vegetable (n = 123, rating from 1 = very high- to 5 = very low in health benefit) by respondents from food service establishments

The frequency distribution of the scores on perceived health benefit of the techniques to prepare *Brassica* vegetables by food service establishment respondents is shown in Figure 3.4. Steaming, boiling, and blanching are perceived to have the highest health benefit, i.e., 22%, 19.5%, and 21% of the respondents give score 1, respectively. Steaming and boiling are also dominant at score 2. Blanching and stir-frying receive lower scores and are thus perceived to have less health benefit.

<u>*Households*</u>

Characteristics of respondents

The majority of the household respondents in both part I (n = 200) and part II (n = 477) of the survey is female and about half of all respondents are housewives (Table 3.3). Furthermore, results from part I of the survey show that 33% out of 200 respondents prepared and consumed *Brassica* vegetables daily in the week prior to the interview. Meanwhile, 27.5% and 22% of the respondents prepared and consumed *Brassicas* three or two times in that week, respectively.

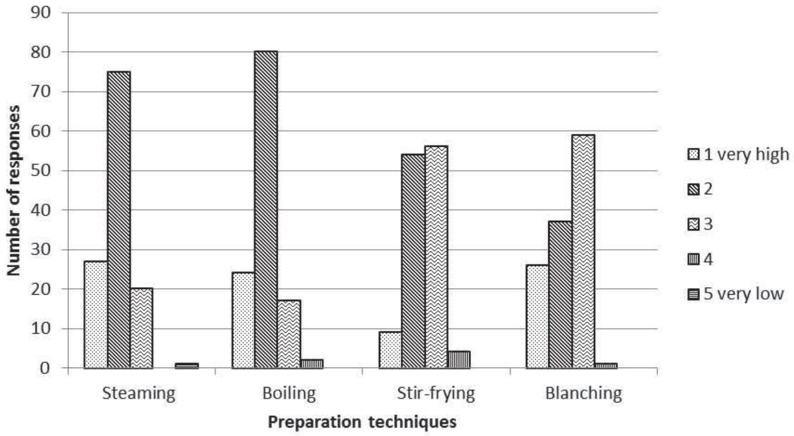

Figure 3.4. Frequency distribution of the scores on perceived health benefit by food service establishments per preparation technique (n = 123, rating from 1 = very high- to 5 = very low in health benefit)

About 85% of the respondents said that the mother is the one who buys and prepares the vegetables. The vegetables are mostly prepared as fresh as possible. Most of the respondents (89%) buy the vegetable in the morning and 71% of the respondents prepare it during that morning, while 18% prepare the vegetable at noon of the same day. In addition, 44% of the respondents buy the raw vegetables from a traditional market nearby the settlement, 34% and 17% of them buy them from mobile or nearby permanent vendors, respectively, and 5% from a supermarket.

Types of frequently prepared Brassica vegetables

The results of part I of the survey show that choysum is the most frequently prepared vegetable among the *Brassicas* (Figure 3.5). At the first and second rank choysum is chosen by 28% and 26% of the 200 and 198 respondents, respectively. Secondly, cauliflower is dominant, accounting for 23% and 25% of the respondents at the first and second rank, respectively, followed by Chinese cabbage. Compared to the food service establishments, white cabbage gets a much lower rank for frequency of preparing *Brassicas*, i.e., 12% to 13.5% of the respondents ranked it first to third. Subsequently, at lower frequency of preparation are broccoli and pakchoy, followed by Chinese kale as the least frequently prepared *Brassica* in the participating households.

Table 3.3. Characteristics of the household respondents in part I and part II of the study

Part	**I**	**II**
Gender (%)		
Female	85	93
Male	15	7
Age (%)		
20-30	21	17
31-40	23	22
41-50	24	35
51-60	24	20
>60	8	6
Occupation (%)		
Housewife	52	59
Self-employee	24	21
Private-employee	10	9
State-employee	2	6
Others	12	5

Preparation techniques

The 477 household respondents of part II of the survey were asked which preparation technique they employed most frequently. Stir-frying is the most frequently applied technique, accounting for 59% of the total responses, followed by boiling (38%). Steaming is hardly employed while blanching and frying are never chosen by the respondents as the most frequently applied technique to prepare *Brassica* vegetables.

Perception on health

The perception of household respondents on the health benefit of consuming *Brassica* vegetables and of the preparation techniques was also assessed. About 43% of the 477 respondents agree with the statement that preparation can reduce the health benefit of the *Brassica* vegetables. Another 50% and 8% of the respondents disagrees and does not know, respectively.

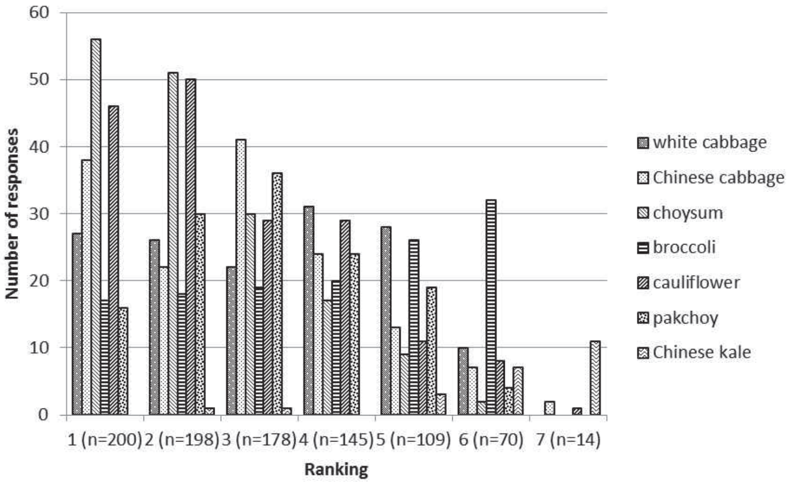

Figure 3.5. Frequency distribution of the ranking of *Brassica* vegetables prepared by households (ranking from 1 = the most- to 7 = the least- frequently prepared; number of responses for each ranking is shown between brackets)

Broccoli is perceived as the most health beneficial *Brassica* by 69% of respondents, followed by choysum (15%). Lower percentages of respondents perceive cauliflower (6%), pakchoy (3%), and Chinese cabbage (3%) as the most health beneficial *Brassica*. Less than 1% of respondents perceives Chinese kale and white cabbage as the most health beneficial *Brassica* and about 3% of the respondents does not know. With respect to preparation technique, boiling and stir-frying are perceived as the most health beneficial techniques, equally chosen by 41% of the respondents. Steaming is perceived as the most health beneficial technique by 13%, about 2% of respondents perceive other techniques (i.e., frying or blanching) as most health beneficial, and the rest (3%) does not know.

Discussion

Food preparation techniques could reflect the diversity in food cultures, and refer to the ways in which humans use food (Kittler and Sucher, 2008). In the Southeast Asian cuisine pre-preparation of the ingredients, such as peeling, cutting/ slicing/ chopping, and grinding, requires a lot of time, but in general the preparation times are short (Van Esterik, 2008). The current study shows that various types of *Brassica* vegetables and types of preparation techniques are commonly employed by food service establishments and households in Semarang, Indonesia.

This survey shows that in the food service establishments white cabbage is the most commonly prepared *Brassica* followed by choysum. The latter is the most commonly prepared *Brassica* by households, while white cabbage is less frequently prepared by them. White cabbage and choysum apparently are the most versatile and familiar *Brassicas*, which can be prepared into various types of dishes or as a complement of other dishes by the food service establishments (Table 3.2). Moreover, white cabbage is the only *Brassica* that is consumed either raw or cooked. Meanwhile, choysum is very popular to be prepared by boiling or sometimes blanching, usually as complement ingredient for noodle or meatball soups.

Boiling and stir-frying are the most dominant techniques to prepare *Brassica* vegetables in both food service establishments and households. These techniques are very familiar for preparing a wide range of *Brassica* vegetables and a large variety of dishes can be produced. However, stir-frying is a relatively seldom applied technique for preparing white cabbage, except for making the *orak-arik* dish (Table 3.2). Most vegetable preparation techniques utilize other ingredients, such as garlic, chili, salt, and any kinds of herbs and other seasonings, as well as meat, seafood, noodle, etc. In practice, these are commonly cooked together in a pan or wok. Eventually, the dish is consumed as a side dish together with staple food, mainly rice.

Interestingly, the less frequently prepared *Brassica*, broccoli is perceived as the most health beneficial *Brassica* followed by choysum. Only a small part of the respondents perceive that white cabbage contributes to the highest health benefit, although it is prepared often. Apparently, the perception on health benefits has no strong association with the frequency of preparing the *Brassica* types. Less than half of the respondents think that preparation can reduce the health benefit of the *Brassica* vegetables. The perception of the health impact of preparation techniques by the food service establishments respondents shows that steaming and boiling are perceived to have the highest health benefit. The health benefit of stir-frying, although being one of the most frequently employed techniques, is perceived to be less than others. On the contrary, for the household respondents, boiling and stir-frying are perceived as the most health beneficial technique, compared to others.

The current study explores a new topic, yet limitations should be acknowledged. The behaviour and health perception related to food preparation may differ from one population group to another and differ over time, as Wahlqvist and Lee (2007) emphasized that locality matters in relation to food culture, in which local conditions must be considered when applying the findings into society. However, the description of preparation techniques by food handlers may reflect a more general behaviour regarding vegetable preparation, particularly in Asia.

Processing or preparation of *Brassica* vegetables changes the amount of health promoting compounds, e.g., glucosinolates, depending on the technique and applied conditions and the type of *Brassica* vegetable (Nugrahedi et al., 2015). The variability of preparation techniques, as observed in the survey, indicates a diversity of potential impact on the glucosinolate amount of *Brassica* vegetables. Some of the techniques might reduce the glucosinolate content significantly while others might retain most of that content. Various mechanisms are taking place during thermal treatment of vegetables, either sequential or simultaneous, which involve (bio)chemical reactions, heat and mass transfer (Dekker et al., 2000). In this respect, the combination of time and temperature plays a critical role in the preparation technique applied.

A short-time stir-frying is supposed to have a limited effect on the reduction of glucosinolates due to the limited time the vegetable is heated and the absence of leaching, but inactivates the hydrolytic enzyme myrosinase. Meanwhile during boiling, leaching is one of the critical factors leading to glucosinolate loss. The habit of consuming the soup liquid will potentially minimize the nutritional implications of this glucosinolate leaching loss. However, boiling during a longer time as well as the habit of reheating leftovers of a dish can considerably reduce the glucosinolate amount due to thermal breakdown.

Further studies are needed to get more detailed insights into actual preparation behaviour and to explain the motivation behind this behaviour for both food service establishments and households. Recent work of Bongoni et al. (2013) introduced 'consumer-oriented food technology' as a research approach for multi-criteria optimization of vegetable quality, including health properties, after domestic preparation. This approach can also be applied towards Asian food preparation practices. Eventually, the results can contribute to optimizing the quality of vegetable products.

Conclusions

The current study reveals that boiling and stir-frying are the most frequently applied preparation techniques to prepare *Brassica* vegetables in food service establishments and households, while other techniques are less frequently performed. Moreover, white cabbage and choysum are the most frequently prepared vegetables among the *Brassica* vegetables. These vegetables are also prepared to more diverse dishes. In terms of perception of health, broccoli is perceived as the healthiest *Brassica* by both households and food service establishments. Furthermore, steaming and boiling are perceived to have high health beneficial effects according to the food service establishment respondents. Meanwhile, for the household respondents boiling and stir-frying are perceived as the most health beneficial preparation techniques.

The obtained survey information on type of vegetables, dishes, and especially preparation techniques were used to evaluate the nutritional implications of the different preparation techniques on the alleged health promoting glucosinolates in *Brassica* vegetable. The results of this study are useful as an approach for developing good and healthy vegetable preparation practices in both food service establishments and households.

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Chapter 4

Quality of cabbage during long-term steaming; phytochemical, texture and colour evaluation



Probo Y. Nugrahedi, Matthijs Dekker, Budi Widianarko and Ruud Verkerk,

Submitted for publication

Abstract

Steaming has been reported to better retain the glucosinolate content in *Brassica* vegetables than boiling. However, there is little information on the glucosinolate content, colour, and texture attributes in *Brassica* vegetables in relation to the duration of steaming. This study investigated the effect of the duration of steaming, which was applied in certain commercial preparation processes, on the glucosinolate content, colour, and texture of white cabbage. Results showed that the total accessible content of glucosinolates increases initially during steaming until 10 min followed by a consistent decline up to 180 min. This observed initial increase is mainly due to the content of aliphatic glucosinolates rather than indole glucosinolates, which tend to decrease from the start of steaming. A mathematic model for the observed behaviour of the glucosinolates, taking into account several mechanisms, is proposed and fitted to the data. The intensity of the green colour of the cabbage slightly increased during the first 15 minutes of steaming followed by a decrease onwards. The hardness showed a continuous decline during the entire steaming duration. The study indicates that steaming up to 10 min could promote the health properties as well as the colour and texture attributes of steamed cabbage.

Keywords glucosinolate, cabbage, steaming, modelling

Introduction

Intake of *Brassica* vegetables has been inversely associated with the risk of lung, colorectal, and prostate cancer (Kristal and Lampe, 2002; Higdon et al., 2007). Several conversion products of glucosinolates (GSs), found almost exclusively in *Brassica* vegetables, are showing biological activities in the human body that are assumed to be responsible for reducing this risk of several cancers (Verhoeven et al., 1997). In intact plant tissue, GSs are stored in separate compartments from the enzyme myrosinase (thioglucosidase EC 3.2.1.147). However, upon the plant tissue disruption, GSs are highly prone to hydrolytic degradation catalysed by the enzyme (Mithen et al., 2000; Fahey et al., 2001). Among the breakdown products of the GSs, isothiocyanates have been reported to inhibit phase 1 and to induce phase 2 enzymes that are beneficial with respect to (pro)carcinogen metabolism and excretion (Traka and Mithen, 2009).

GSs are water-soluble compounds that may leach into cooking water during vegetable preparation. For example, boiling of *Brassica* vegetables results in 25%–75% decreases in total GS content (Nugrahedi et al., 2015). Cooking methods that use less water, such as steaming and microwaving, have shown to reduce GS losses (Vallejo et al., 2002; Verkerk and Dekker, 2004; Rungapamestry et al., 2006; Song and Thornalley, 2007). Several types of processing of *Brassica* vegetables have been studied and many have a pronounced impact on the concentration of GSs and their corresponding isothiocyanates (Verkerk et al., 2009). The observed effects can be explained by multiple mechanisms such as i) myrosinase inactivation, ii) cell lysis and leaching of GSs, breakdown products, and myrosinase in the cooking water, and iii) thermal degradation of GSs (Nugrahedi et al., 2015).

Often, these studies describe various cooking procedures based on the dietary habits and cuisines in the Western society. While studies on Asian preparation methods of *Brassica* vegetables and the effects on phytochemical content have been underexposed. Probably the processes and underlying mechanisms responsible for GS losses are similar for different cuisines, however the extend of losses can vary based on different characteristics of Asian vegetable preparation methods and the use of different vegetable-based products in the Asian cuisine.

Steaming has been reported to better retain the GS content in *Brassica* vegetables than boiling and blanching (Rungapamestry et al., 2006; Miglio et al., 2008; Volden et al., 2009b). However, steaming of *Brassica* vegetables extensively for long times, could result in substantial losses of GSs differently from short steaming processes reported in literature thus far. An example product using long-term steaming is cabbage roll, a typical Asian dish made from shortly blanched leaves of white cabbage that are folded and rolled, and subsequently steamed. Steamed cabbage roll is usually served as a kind of dim sum, which includes also e.g., boiled potato and tofu. The product is produced and sold generally by street- or mobile-vendors (Tan, 2002). During selling, the rolls can be steamed constantly for more than two hours, depending on the selling rate, on a very low flame level. To our knowledge, there is little information on the behaviour of GSs in *Brassica* vegetables in relation to the long duration of steaming (Song and Thornalley, 2007; Verkerk et al., 2010).

In order to understand and simulate the changes of concentration of GSs as affected by processing, previous studies employed kinetic modelling to describe and predict these changes. Leaching and thermal degradation are the main mechanisms affecting GS changes since myrosinase is a thermolabile enzyme and is inactivated early in most thermal processes (Hennig et al., 2012; Sarvan et al., 2012; Sarvan et al., 2014).

Next to these effects of the process on the GS content and subsequent health value, colour and texture are the important sensorial attributes perceived by the consumers to evaluate the quality of both fresh and cooked vegetables (Jackman and Stanley, 1995; Nisha et al., 2004; Miglio et al., 2008). The degree of greenness is an important colour quality attribute of thermally processed green vegetables. The changes of hardness, softness, or firmness can be expressed as important textural quality attributes of vegetables .

The present study aims to investigate the effect of a long duration of steaming on the GS content, colour, and texture of the cabbage roll. In addition, the changes of GSs during steaming are also mathematically modelled to gain insight in the important mechanisms involved. In the societal context this study will be beneficial to know the changes of the health-promoting and physical quality attributes of vegetable products and to optimise real preparation methods commonly employed by especially Asian food service establishments.

Materials and methods

Sample preparation

Two batches of raw white cabbages (*Brassica oleracea* L. *Capitata*) were collected from one local supplier in Semarang, Indonesia on two consecutive days. Six heads of white cabbage were used in each experimental batch. Damaged outer leaf layers of the cabbage heads were removed. Leaf layers of the white cabbage from the surface until about half diameter of the head were taken, washed by running tap water and drained.

Blanching and steaming

The preparation method of the cabbage rolls was performed in triplicate by mimicking the common practice of the small-scale food service establishments in Semarang, Indonesia. Heat was provided by using a liquefied petroleum gas stove (Rinnai RI-602E, gas consumption = 2.9 kWh^{-1}). Briefly, leaves of white cabbage were blanched in the distilled water at the ratio of 1:6 (w/v) for 3 min in an open aluminium pan. Then, each leaf was rolled manually resulting in a cabbage roll at the diameter of about 3 cm and the length of about 7 cm. Each roll was made from about 35 g fresh leaves. Each batch of processing took about 5 minutes for rolling all the cabbage leaves.

Subsequently, all rolls were steamed in a closed steaming pan containing boiling water for 180 min, comparable with the artisanal procedure often applied in Asian food service establishments. The rolls were arranged in two layers in a way that the steam can still reach the lid. The flame was maintained at the lowest level during steaming resulting in gentle boiling of the water. The number of the rolls in the steaming pan was twice of the number of samples needed for each batch analysis and sample rolls were taken randomly only from the top layer of rolls at certain time-points of steaming until 180 min. The analyses of GSs, colour, and texture were performed on separate rolls.

Glucosinolate analysis

Three leaves of raw, or rolls of blanched and steamed cabbage were chopped into pieces and directly frozen in liquid nitrogen followed by frozen grinding in a stainless steel blender (Waring 2-speed EW 04242-11) and storing at -20°C until lyophilisation for 5 days (Heto Power Dry LL1500, Thermo Scientific). The sample of the steaming water was prepared by freezing 10 mL of the water in liquid nitrogen followed by storing it in the freezer at -20°C until further analysis.

Extraction and desulphation

The extraction method of GSs in the cabbage used hot methanol as the solvent followed by on-column desulphation as described by Verkerk et al. (2001) with slight modifications, i.e., fivefold downscaling the mass and volume during extraction and centrifuging at 3500 ×g following incubation.

<u>HPLC analysis</u>

HPLC set-up used a Shimadzu LC 10 Avp with a manual injection system (Rheodyne 7725i). A UV Vis detector (SPD 10 Avp) was performed at a wavelength of 229 nm. The de-sulpho-GSs were separated by using a GraceSmart RP-18 5µ column. The elution was performed by the gradient system of water containing 0.05% tetra-methyl ammonium chloride and acetonitrile/water (40:60, v/v) containing 0.05% tetra-methyl ammonium chloride. The flow rate, total elution time, and gradient flow followed Verkerk et al. (2001). The GSs were identified by comparing with standards of sinigrin and comparison with the chromatograms of reference materials (broccoli, cauliflower, red cabbage, radish, and *Brussels* sprouts) with known GS profiles, as well as confirming with data from literature. Each GS was quantified using the published response factors against glucotropaeolin as internal standard. The total GS concentration was determined by adding up all individual GS (Verkerk et al., 2001).

Colour

Colour of the samples was recorded as the colour coordinates $L^*a^*b^*$ by a chromameter CR 400 (Konica Minolta). The instrument was calibrated before each series of measurements using the standard white tile (L^* = 97.39, a^* = 0.02, and b^* = 1.69). Colour of the cabbage roll was measured at the surface of the roll laid down on the white tile while the fresh sample was measured as leave. Triplicate samples of each treatment were taken from each experimental batch. Each roll was measured in duplicate at different locations. The ratio $-a^*/b^*$ was calculated to represent the greenness of the cabbage (Tijskens et al., 2001).

Texture

Six cabbage rolls of each treatment were taken from each experimental batch. Each roll was placed on the base table and measured once at the middle of its longitudinal axis. A texture analyser (TAPlus, Lloyd) was used to examine the hardness value, i.e., the peak force of the first compression cycle during the measurement. The analysis was performed by a compression test to the cabbage roll until 25% of the original thickness by using a 25 mm cylinder probe and the speed at 5 mm/s (modified from Christiaens et al., 2011). The percentage of softening was calculated as:

Softening (%) = (1 - (maximum force of steamed sample at t_n / maximum force of steamed sample at t_0)) * 100% (modified from Miglio et al., 2008)

Kinetic modelling of GS content during steaming

The changes of GS content of the cabbage rolls during steaming can be influenced by the following mechanisms:

1. Cell lysis;
2. Enzymatic hydrolysis by endogenous myrosinase;
3. Myrosinase inactivation;
4. Increase in extractability of GSs;
5. GS thermal breakdown;
6. Steam condensation on the rolls;
7. GS leaching to the condense water on/in the rolls;
8. Condense water containing GSs dripping from the rolls.

The mechanisms 1, 5, and 7 are modelled according to the equations as previously published for general thermal processing of *Brassica* vegetables by Sarvan et al. (2012). The changes due to mechanisms 2 and 3 are assumed to be negligible due to the fast inactivation of myrosinase during the relatively short heat-up phase during the blanching pretreatment of the cabbage.

Heating up of the cabbage rolls

The measurements of the temperature of the cabbage rolls could be described by an asymptotic increase until the final temperature of 373 K was reached, by the following equation:

$$\frac{\mathrm{d}T}{\mathrm{d}t} = k_T \cdot (T_{final} - T) \qquad (1)$$

In Figure 4.1 the vegetable tissue is shown schematically as it is build-up of the different compartments and how they are described by the model equations.

Increased extractability

The increased extractability (mechanism 4) is assumed to be caused by the fact that a part of the GSs is located in a fraction of the vegetable tissue that is not available for the extraction procedure. The lysis of cells in this fraction (expressed as a fraction of the total vegetable weight: $F_{GS\text{-}trapped}$) is assumed to follow the same kinetics as the cell lysis of the other cells, in order to not have an additional parameter to be estimated from the data. So, when cell lysis occurs the measured GS content is increasing due to this mechanism, a phenomena that is often described in literature (Verkerk and Dekker, 2004; Gliszczyñska-šwiglo et al., 2006; Oerlemans et al., 2006; Miglio et al., 2008).

$$F_{GS\text{-}trapped} = M\_veg_{inaccessible}/M\_veg_{total} \qquad (2)$$

$$C_{GS,\ measured} = (1\text{-}\ F_{GS\text{-}trapped})\cdot C_{GS,\ total} \qquad (3)$$

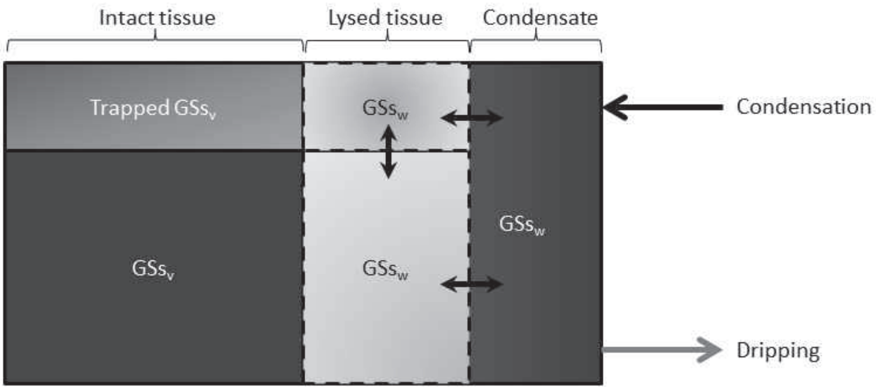

Figure 4.1. Schedule of the vegetable tissue during steaming consisting of different compartments. When analysing the GS concentration in the vegetable samples the trapped GSsv are not detected.

Steam condensation and dripping

Steam will condensate on the cabbage rolls and part of this condensate will be absorbed by the rolls (mechanism 6). The amount of condensed steam on/in the cabbage rolls is measured by weighing the rolls during the three hour steaming process. It was observed that the weight gradually increased until a maximum of on average 18% weight gain relative to the starting weight of the vegetable rolls (data not shown). This condensate absorption by the rolls is modelled by the following equation:

$$\frac{\mathrm{d}M_C}{\mathrm{d}t} = k_{wg} \cdot (M_{C,final} - M_C) \qquad (4)$$

Also the lysed part of the vegetable tissue will increase in time (Sarvan et al., 2012) and can be described by:

$$\frac{\mathrm{d}M_L}{\mathrm{d}t} = -\frac{\mathrm{d}F_i}{\mathrm{d}t} M_{v,0} = k_l \cdot F_i \cdot M_{v,0} \tag{5}$$

The mass total 'free' water is the sum of the condensate mass and the lysed cell mass:

$$M_w = M_C + M_L \tag{6}$$

The concentration of GSs in the total free water mass on/in the cabbage rolls is described by four processes:

1. The increase by GSs leaching from lysed accessible and inaccessible (trapped GSs) cells (*L*)
2. The decrease by the condensation of steam (free of GSs) and the drip-flow of condensate, containing GSs (*D*)
3. The decrease by the increasing absorbed condensate mass on/in the rolls (*C*)
4. The decrease by thermal breakdown of GSs present in the condensate (*B*)

$$\frac{\mathrm{d}C_{g,w}}{\mathrm{d}t}\Big|L = \frac{k_l \cdot F_i \cdot M_{v,0}}{(1 - F_{GS-trapped})} \cdot \frac{\left(C_{g,v} - C_{g,w}\right)}{M_w} \tag{7}$$

$$\frac{\mathrm{d}C_{g,w}}{\mathrm{d}t}\Big|D = -F_{con} \cdot \frac{C_{g,w}}{M_w} \tag{8}$$

$$\frac{\mathrm{d}C_{g,w}}{\mathrm{d}t}\Big|C = -\frac{C_{g,w}}{M_w} \cdot \frac{dM_C}{dt} = -\frac{C_{g,w}}{M_w} \cdot k_{wg} \cdot (M_{C,final} - M_C) \tag{9}$$

$$\frac{\mathrm{d}C_{g,w}}{\mathrm{d}t}\Big|B = -k_{d,w} \cdot C_{g,w} \tag{10}$$

The resulting change in time of this GS concentration in the water phase is the sum of these four equations.
The GS concentration in the intact vegetable cells (accessible and trapped), $C_{g,v}$, is described by a first order degradation reaction (Sarvan et al., 2012).

<u>*Measured GS concentration in the cabbage rolls*</u>

The measured GS concentration in the cabbage rolls is the sum of the measurable amount of GSs in the intact vegetable cells plus the GSs in the lysed vegetable cells and in the condensate in/on the rolls divided by the total mass of the rolls, which is the mass of cabbage plus the mass of condensate :

$$C_{g,measured} = \frac{\{(1 - F_{GS-trapped}) \cdot F_i \cdot C_{g,v} \cdot M_{v,0}\} + \{C_{g,w} \cdot ((1 - F_i) \cdot M_{v,0} + M_C)\}}{(M_{v,0} + M_C)} \tag{11}$$

The model calculates the concentration of GSs expressed as µmol/100 g FW. Meanwhile, the samples are measured as µmol/100g DW. To convert the FW concentrations to the DW the weight increase of the additional condensation water is taken into account to convert the model predictions into a concentration expressed as µmol/100 g DW. The initial DW% of the vegetable mass (so, before the uptake of condensate) is assumed to be 10% (w/w). Additional soluble solid loss from the vegetable during steaming was assumed to be negligible.

Data analysis

Numerical integration of the equations and parameter estimation of the rate constants followed the procedure described by Sarvan et al. (2012) by using software package Athena Visual Workbench (www.athenavisual.com).

Results and Discussion

Glucosinolates of raw white cabbage

Five GSs were identified in the raw white cabbage: glucoiberin, glucoraphanin, gluconapin, glucobrassicin, and 4-methoxy-glucobrassicin (Table 4.1). The aliphatic GSs were the most dominant with 60% of the total concentration, and glucoraphanin as the major constituent at 37% of the total GSs. Indoles accounted for the other 40% with glucobrassicin as the most prominent (37% of total GSs). This GS composition is comparable to previous studies on white cabbage, although some studies report the sinigrin content of white cabbage to be higher compared to the glucoraphanin content (Ciska and Kozłowska, 2001; Ciska and Pathak, 2004; Wennberg et al., 2006; Fuller et al., 2007; Song and Thornalley, 2007). The differences in the sinigrin/glucoraphanin content described in literature could be due to the differences in the cultivar and cultivation conditions that are known to affect the GS profile and content substantially (Ciska et al., 2000; Krumbein et al., 2005; Kang et al., 2006).

Table 4.1. Glucosinolate concentration (µmol / 100 g DW) in white cabbage

Glucosinolate	*Raw*	*Blanched*
Glucoiberin	246.49 ± 43.72	337.96 ± 86.89
Glucoraphanin	475.90 ± 86.11	566.90 ± 162.10
Gluconapin	42.21 ± 7.27	91.50 ± 28.98
Glucobrassicin	474.17 ± 239.54	497.86 ± 133.45
4-Methoxy-glucobrassicin	36.39 ± 6.57	45.61 ± 19.28
Total GSs	1275.15	1539.82

Values are presented as means ± standard deviations (n = 6)

Glucosinolates of cabbage roll

Blanching of the cabbage leaves for 3 minutes, as first step in the procedure of cabbage roll preparation, led on average to an increase of 21% of total GSs compared with the raw cabbage (Table 4.1). Both, the processing conditions and the *Brassica* vegetable type can influence the behaviour of GSs upon heating. Some studies reported that boiling can increase the GS concentration in *Brassica* vegetables. Usually this is observed at short heat treatments, and ascribed to a higher extractability of GSs in the boiled tissues (D'Antuono et al., 2007; Fuller et al., 2007; Rungapamestry et al., 2008). However, extensive boiling was generally reported to considerably reduce GS content of *Brassica* vegetables even to more than 50% of the raw material (Vallejo et al., 2002; Gliszczyñska-šwiglo et al., 2006; Song and Thornalley, 2007; Miglio et al., 2008). Extensive leaching and thermal degradation of GSs was indicated as the major contributors to the losses (Volden et al., 2009b; Francisco et al., 2010; Nugrahedi et al., 2015).

The total GS content after blanching was relative stable during the first 10 min of steaming (Figure 4.2). Subsequent steaming of the cabbage rolls led to a gradual decrease of 66% in total GS content after 180 minutes. A marked difference in behaviour was observed between aliphatic and indole GSs; about 54% of total aliphatic GSs were lost, while more than 90% of total indole GSs of the blanched cabbage rolls was lost after steaming for 180 min. It can be assumed that most of the hydrolytic enzyme myrosinase had been inactivated by the blanching step prior to steaming, so the decrease in GS content will be mainly caused by leaching and thermal degradation. Verkerk et al. (2010) reported that the total GS content in steamed broccoli, as compared with the untreated broccoli, was up to 55% higher after about 10 min and approximately 4% lower levels after 30 min of steaming. Meanwhile, Song and Thornalley (2007) reported no significant losses of total GSs in broccoli, green cabbage, cauliflower, and *Brussels* sprouts over 20 min of steaming. Moreover, after 30 min steaming of broccoli substantial losses of total indole GSs (55%) and less loss of total aliphatic GSs (8.5%) were observed (Verkerk et al., 2010), confirming the present observation that indole GSs are less retained during steaming than the aliphatic GSs.

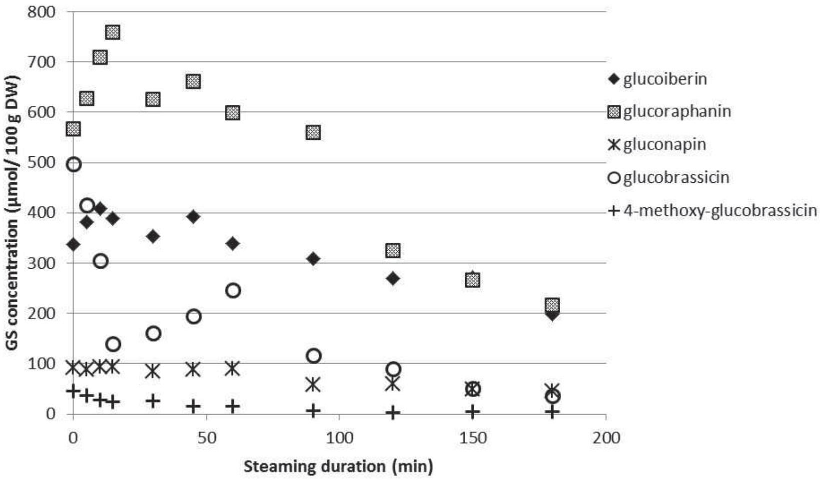

Figure 4.2. Glucosinolate concentration (µmol/ 100 g DW) along the production of cabbage roll (n = 6)

One of the possible causes of GS losses during longer steaming duration could be thermal degradation of GSs (Verkerk et al., 2010). Nevertheless, the heating rate of the vegetable tissue is generally lower in steaming compared to boiling. Moreover, since there is no direct contact between the vegetable tissue and a large pool of water, steaming is expected to result in less leaching compared to boiling (Nugrahedi et al., 2015). Nevertheless, losses by leaching into the condensed steam followed by drip loss can still be an important loss mechanism in (long-term) steaming. Shorter steaming times have been reported to have benefits over longer ones in terms of better retaining the GSs (Galgano et al., 2007; Miglio et al., 2008).

Mathematical modelling of glucosinolate changes during steaming

<u>*Parameter estimation for glucoraphanin*</u>

The complete model description as described above consists of many parameters. In order to reduce the number of parameters to be estimated from the current experimental data, some parameter estimates have been taken from literature (Volden et al., 2008b; Sarvan et al., 2012; Sarvan et al., 2014). Table 4.2 shows the parameter estimates during the steaming experiment. Two parameters (k_{Temp} and k_{wg}) were estimated from the measurements of the temperature and weight increase of the rolls during the steaming experiments. Three parameters were estimated from the experimental GS data, i.e., $C_{g,v}$, $F_{GS\text{-}trapped}$ and F_{con}.

Table 4.2. Parameter estimates during the steaming experiment

Parameter	*Value*	*SD*	*Reference*
$k_{L,100°C}$	0.11 min^{-1}		Sarvan et al. (2012)
Ea_k_L	53 kJ/mol		Sarvan et al. (2012)
$k_{dv,100°C\ raphanin}$	5.9 10^{-3} min^{-1}		Sarvan et al. (2014)
$Ea\_\ k_{dv,100°C\ raphanin}$	93 kJ/mol		Sarvan et al. (2014)
$k_{dw,100°C\ raphanin}$	6.8 10^{-3} min^{-1}		Volden et al. (2008b)
$Ea\_\ k_{dw,100°C\ raphanin}$	93 kJ/mol		Sarvan et al. (2014)
k_{Temp}	0.12 min^{-1}		
k_{wg}	0.03 min^{-1}		
$C_{g,v}$ (µmol/100 g DW)	637	34	
$F_{GS\text{-}trapped}$ (-)	0.22	0.09	
F_{con} (g/min)	0.028	0.030	

Figure 4.3 shows the experimental data with the model description for glucoraphanin. The model fits the observed concentration profile of glucoraphanin during the steaming duration. The estimated values of the condensate/drip flow seems to be very low. Based on measurements of water evaporation rate (around 1.5 g/min) a much higher value would be expected. Not all the evaporated water is expected to condensate on the vegetable rolls since most of the steam will condense on the wall and lid of the pan. But the value of F_{con} corresponds to only 2% of the evaporated water flow, which is lower than expected. This is most likely caused by the fact that the assumption on diffusion limitation is not realistic for the size of rolls. This means that the estimated drip flow actually represents a combination of this diffusion limitation and condensate/drip flow.

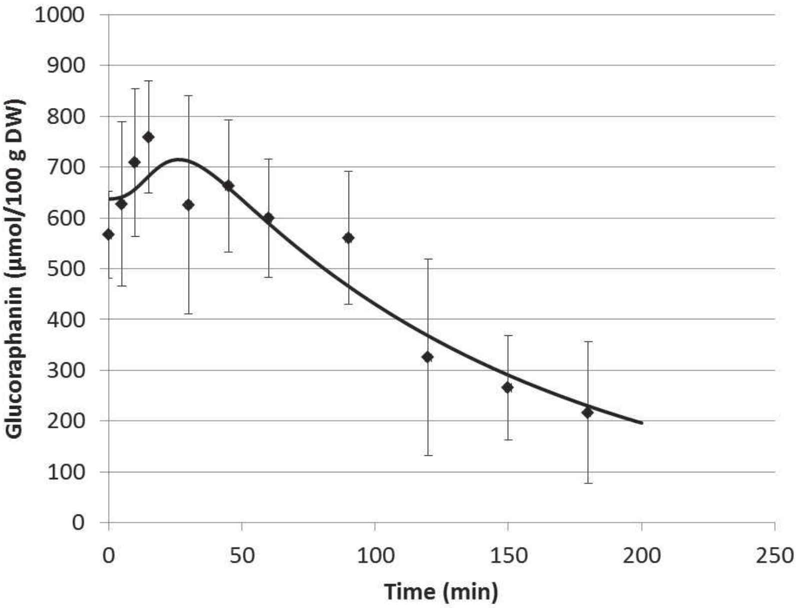

Figure 4.3. Experimental data (symbols) and model description (line) of the glucoraphanin content during long term steaming.

Colour

Figure 4.4 shows that the greenness level -*a**/*b** increased steadily after blanching followed by steaming for up to 15 min. Subsequently, a steady decrease was observed. Possibly, the increase of greenness level is affected by physical changes in the vegetable matrix, the conversion of non- or less-coloured precursor into more visible green intensity, or a contact between the enzymes and the chlorophyll precursor compounds present in different organelles (Tijskens et al., 2001).

The high temperature during prolonged steaming of cabbage roll causes considerable losses of greenness level. Chlorophylls are susceptible to thermal degradation during processing (Von Elbe and Schwartz, 1996). Moreover, leaching of the liberated colouring compounds could occur (Tijskens et al., 2001).

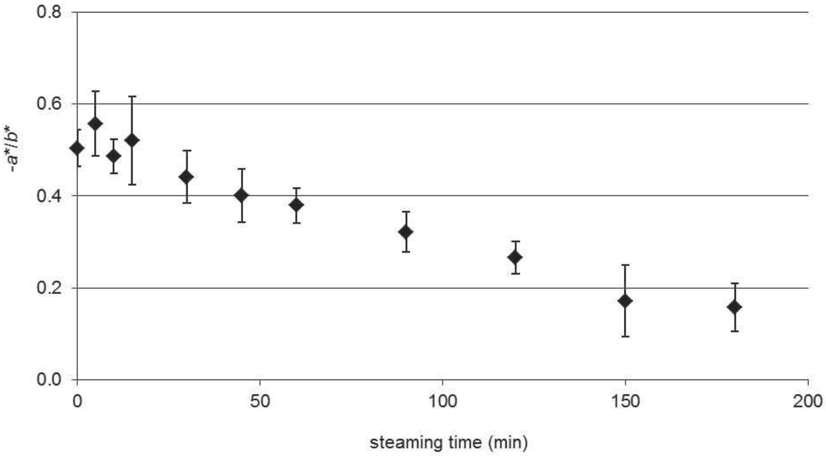

Figure 4.4. Greenness level (mean ± SD) of cabbage roll during steaming (n = 18)

Texture

Figure 4.5 shows the hardness values of the cabbage roll decreased consistently during steaming. Cooking of vegetables or fruit is known to result in an initial loss of instrumental firmness due to membrane disruption and the associated loss of turgor. Additional softening occurs as a result of an increase in the ease of cell separation (Haard and Chism, 1996). Furthermore, cooking can reduce the strength of cell adhesion in many vegetables and fruit through depolymerisation of pectic polysaccharides (Waldron et al., 1997).

The percentage of softening of blanched sample with respect to the raw increased from 20% into 75% at 5 and 180 min, respectively. Previous studies also reported that the degree of softening due to heat treatment is varied depending on the types of both preparation and *Brassica* vegetable (D'Antuono et al., 2007; Miglio et al., 2008).

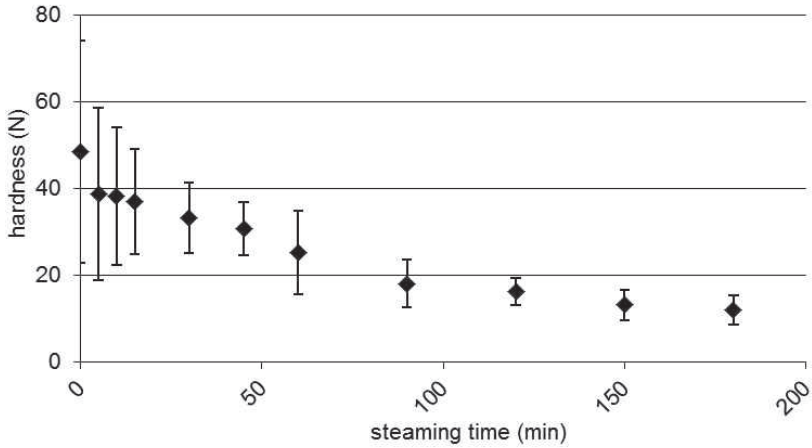

Figure 4.5. Hardness (mean ± SD) of cabbage roll during steaming (n = 18)

Conclusions

The behaviour of glucosinolates (GSs) of white cabbage during long-term steaming has been studied. The total GSs increase after blanching for 3 min followed by steaming for 10 minutes. After this initial phase, a decline of total GSs is observed during the long-term steaming up to 180 min. This behaviour is attributed mainly to the aliphatic GSs. The indole GSs tend to decrease during the whole process of steaming. Glucoraphanin and glucobrassicin are the two dominant GSs found in the cabbage. The changes in the GS content of the cabbage rolls could be described by a mechanistic model taking into account several mechanisms that affect the GSs. The greenness colour of cabbage roll slightly increases during the initial phase of steaming followed by a decrease onwards. The hardness value shows a continuous decline during long-term steaming. The study indicates that long-term steaming as is done in food service establishments in Indonesia causes considerable loss of GSs as well as the colour and texture attributes of cabbage roll. Alternative preparation/storage conditions of these products can improve these quality attributes.

Acknowledgments

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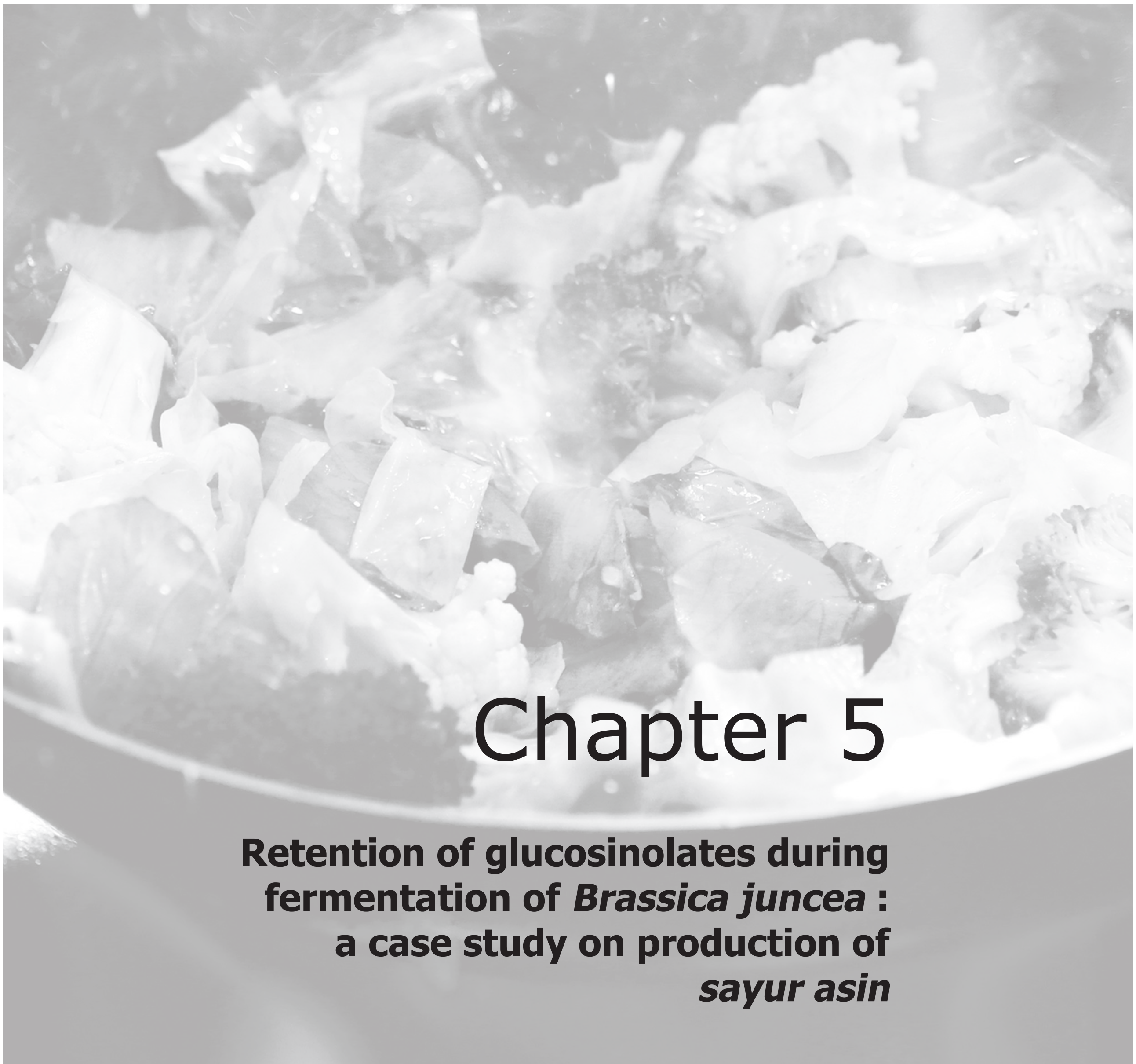

Chapter 5

Retention of glucosinolates during fermentation of *Brassica juncea* : a case study on production of *sayur asin*

Probo Y. Nugrahedi, Budi Widianarko, Matthijs Dekker,
Ruud Verkerk, and Teresa Oliviero

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Abstract

Fermentation can reduce the concentration of health-promoting glucosinolates in *Brassica* vegetables. The endogenous enzyme myrosinase is hypothesized to mainly responsible for the degradation of glucosinolates during fermentation. In order to retain glucosinolates in the final fermented product, the role of myrosinase activity during the production of *sayur asin* was investigated. *Sayur asin* is a traditionally fermented product of Indian mustard (*Brassica juncea*) commonly consumed in Indonesia. It is prepared by a spontaneous fermentation of withered (sun-dried) *Brassica juncea* leaves. The leaves of *Brassica juncea* contain a substantial amount of the aliphatic glucosinolate sinigrin. Three withering methods were investigated to obtain *Brassica juncea* leaves with different myrosinase activities prior to fermentation. Results show that withering by oven at 35 ˚C for 2.5 h and by microwave at 180 W for 4.5 min reduced myrosinase activity by 84 % and 74 %, respectively. Subsequently, sinigrin was not detectable in the leaves after 24 h of incubation in the fermentation medium. However, withering by microwave for 2 min at 900 W inactivated myrosinase completely and produced *sayur asin* with a sinigrin concentration of 11.4 µmol/10 g dry matter after seven days of fermentation. This high power-short time pretreatment of *Brassica juncea* leaves contributes to the production of *sayur asin* containing significant levels of health-promoting glucosinolate. In this study, the effect of myrosinase activity during *Brassica* fermentation was quantified, and optimized production methods were investigated to retain glucosinolate in the final product.

Keywords glucosinolate, myrosinase activity, fermentation, *Brassica juncea*

Introduction

Glucosinolates (GSs) are a group of β-thioglucoside N-hydroxysulphates with a sulphur linked β-D-glucopyranose moiety and side-chain group. GSs are secondary metabolites derived mainly from one of certain amino acids, such as methionine, tryptophan, and phenylalanine. Depending on side-chain group, GS can be classified either as aliphatic, aromatic, or indole (Halkier and Gershenzon, 2006; Verkerk et al., 2009; Clarke, 2010).

Reviews of epidemiological studies reported inverse associations between intake of *Brassica* vegetables and the risk of certain cancers (Cartea and Velasco, 2008; Herr and Büchler, 2010). Isothiocyanates, one of the GS breakdown products, are assumed to lower the risk of cancer by inhibiting phase 1 and inducing phase 2 enzymes during carcinogen metabolism (Traka and Mithen, 2009). Upon damage of plant tissue, GSs are highly prone to hydrolysis catalysed by myrosinase (β-thioglucosidase EC 3.2.1.147), an enzyme that occurs in *Brassica* vegetables (Bones and Rossiter, 2006; Verkerk et al., 2009).

Fermentation is a processing method commonly performed on milks, meats, and vegetables (Caplice and Fitzgerald, 1999). Fermentation of *Brassica* vegetables involves the growth and metabolic activity of lactic acid bacteria, either spontaneously or starter-induced, to produce fermented products (Tolonen et al., 2002). Previous studies reported that fermentation considerably reduces the concentration of GSs in *sauerkraut* (Ciska and Pathak, 2004; Martinez-Villaluenga et al., 2009) and radish *kimchi* (Kim and Rhee, 1993).

The mechanisms underlying GS changes in *Brassica* vegetables during fermentation are less investigated than other processing methods, such as boiling, steaming, and microwave processing (Ruiz-Rodriguez et al., 2008; Nugrahedi et al., 2015). Several factors can influence the loss of GS during fermentation, such as the myrosinase activity, lactic acid bacteria growth and activity, low pH, and sodium chloride concentration (Tolonen et al., 2002; Martinez-Villaluenga et al., 2009; Sarvan et al., 2013). *Sayur asin* is a traditional fermented product of Indian mustard (*Brassica juncea*) commonly produced in some regions in Indonesia (Puspito and Fleet, 1985) and other Asian countries with their own local names (Lee, 1997). The product is prepared by a spontaneous fermentation for 7 days of withered (sun-dried) Indian mustard leaves in the addition of salt followed by rubbing (Puspito and Fleet, 1985). Either starch water of boiling rice or coconut water can be used as the fermentation medium. In the preliminary study, it was shown that during the production of *sayur asin* sinigrin concentration was reduced to 1.3% after three days of fermentation (Nugrahedi et al., 2013). The loss of GS is probably attributed to hydrolytic activity of myrosinase. The myrosinase activity is mainly ascribed to the endogenous myrosinase present in the leaves, although microrganisms can also show a myrosinase-like activity (Shapiro et al., 1998; Oliviero et al., 2014b).

This study aims to retain the GS in Indian mustard during the *sayur asin* production and to study how the myrosinase activity affects the GS concentration during the fermentation. For this purpose, three withering methods were applied to produce Indian mustard leaves with different myrosinase activities prior to fermentation, namely by oven at 35 ˚C and by microwave at 180 W and 900 W. Upon withering the samples were fermented for 7 days and the GS concentration and the myrosinase activity were monitored. Previous studies reported the diversity of microbes in traditionally fermented sayur asin (Puspito and Fleet, 1985) and mustard products (Chao et al., 2009). Since the present study modifies the production method of *sayur asin*, the microbiological profile of sayur asin was also investigated.

Material and methods

Preparation of inoculum and fermentation medium for sayur asin

Since the withering treatments will greatly reduce the number of natural lactic acid bacteria on the leaves, a standard inoculum was prepared for all three treatments, by taking an aliquot of coconut water of a 5 days fermented *sayur asin* previously withered in the oven at 35 ˚C. The aliquot was inoculated in the coconut water (Omega & More, Witsenburg Natural Products BV) at 1% (v/v) to achieve about similar number of total lactic acid bacteria that is reached at the beginning of fermentation of a traditionally withered *sayur asin* (i.e., approximately 6 log CFU/ml).

Preparation of sayur asin

Raw Indian mustard was obtained from a local supplier (Oriental Webshop, Duiven, The Netherlands). Indian mustard leaves were separated, washed, drained and randomly divided into portions of 200 g (3 to 4 leaves). There were three withering methods performed, i.e., by an oven at 35 ˚C for 2.5 h to mimic the traditional process, by microwave (MW) at a power of 180 W for 4.5 min to reduce the myrosinase activity, and by MW at 900 W for 2 min to inactivate the myrosinase activity. Hence, the withered leaves containing different levels of myrosinase activity were obtained. Then, salt was added to the withered vegetables (2.5%, w/w) and rubbed gently by hand, similar to the traditional process. Subsequently, the leaves were immersed in coconut water containing 1% (v/v) of the standard inoculum, at a ratio of 1:5 (w/v). Each product was packed in 360 mL glass jar and incubated in the dark at a room temperature of 22 ˚C for 7 days. Each treatment was carried out in triplicate.

Sample preparation

Half of the amount of the vegetable and of the medium in the jar were taken aseptically followed by a quick freezing in liquid nitrogen, while the remaining vegetable and medium of the sample was mixed thoroughly with a stomacher for further microbiological analysis. The raw, withered, and fermented vegetables as well as the fermentation medium were frozen in liquid nitrogen followed by lyophilisation. Subsequently, the vegetables were grinded and stored at -22 ˚C until further analyses of glucosinolate (GS) and myrosinase activity. These analyses were also performed to the fermentation medium.

Myrosinase activity analysis

The myrosinase activity was determined by a coupled enzymatic procedure followed by spectrophotometry measurement (Van Eylen et al., 2006), in which the produced glucose, from the reaction of native myrosinase and a standard GS, was measured. A 50 mg lyophilized sample was mixed overnight in 140 mL of 50 mM potassium phosphate buffer (pH 7.0) at 15 ˚C. On the second day, the plant matrix was removed from the myrosinase solution by centrifugation at 2670 g for 10 min followed by clarification (folded filters Grade 595 ½- 4-7 µm, Whatman). Subsequently, 2 mL of this solution was filtered (cut-off 30 kD Amicon Ultra-4 Millipore) from the molecules smaller than myrosinase with the aid of centrifugation at 4000 g for 10 min. The concentrated enzyme was dissolved in 500 µL of potassium phosphate buffer. Eventually, the myrosinase activity was measured by a D-glucose enzyme kit (Enzyplus EZS 781+). The reaction mixture consisted of a water solution containing 0.05 g/L of magnesium chloride and 1 g/L ascorbic acid, a ATP/ $NADP^+$ solution, buffer solution, hexokinase/ glucose-6-P-dehydrogenase solution and 50 µL sample solution containing the myrosinase, and sinigrin 30 mg/mL solution. The formation of NADPH was followed by a spectrophotometer (Cary UV 50) at 340 nm for 7 min. To quantify myrosinase activity, an external calibration was determined by following the same procedure for the samples analysis. In the reaction mixture 50 µL a standard myrosinase solution instead of the myrosinase sample extract was added. Activity was expressed as unit/g dry material (DM) where, one unit produces 1.0 µmol glucose per minute from sinigrin at pH 6.0 at 25 ˚C.

Glucosinolate analysis

Extraction of GSs with hot methanol (70%), containing an internal standard of glucotropaeolin, followed by on-column desulphation and analysed by high performance liquid chromatography was done in duplicate according to the procedure described by Sarvan et al. (2013). The de-sulpho-GSs were separated by using the Lichrospher100 column (Merck RP-18, 5 µm) with an attached LiChroCART guard column (RP-18, 4×4 mm) (Merck, Darmstadt) at a flow rate of 1 mL/min. The elution was performed by the gradient system of water and acetonitrile. Detection was performed with a DAD detector (Spectra System UV 6000 LP) at a wavelength of 229 nm. The GSs were identified by the spectra as well as comparing with the external standard of sinigrin and internal standard of glucotropaeolin.

Microbiological analyses

Samples of the fermenting liquid and vegetables were collected from the jars for counting of total bacteria, lactic acid bacteria, yeasts, and *Bacillus cereus* by the standard spread plate method. Total colony counts, lactic acid bacteria, *Bacillus cereus*, and yeasts were counted on Plate Count agar (PCA, Oxoid), deMan-Rogosa-Sharpe agar (MRSA, Merck), Mannitol Yolk Polymyxin agar base (MYP, Merck) plus egg yolk emulsion and *Bacillus cereus* selective supplement, and Oxytetracycline Glucose Yeast Extract agar (OGYE, Oxoid), respectively. Plates of PCA, MYP, and OGYE were aerobically incubated at 35 ˚C for 2 days, at 32 ˚C for 1 day, and at 25 ˚C for 5 days, respectively. Plates of MRS were anaerobically incubated at 35 ˚C for 3 days. The colonies that developed on each plate were counted.

Statistical analysis

Effects of withering methods, namely the oven, MW 180, and MW 900, on GS concentration, total lactic acid bacteria, and total colony count during the production of *sayur asin* were analysed by one way Anova and Duncan's multiple range test at the 5% significance level (IBM SPSS Statistics version 20, U.S.). Each sample was produced in triplicate and the GS analysis was performed in duplicate.

Results and discussion

Myrosinase activity

The effect of withering methods on myrosinase activity

Raw leaves of Indian mustard showed a myrosinase activity of 162 ± 56 units/g DM. The effect of the three withering methods on myrosinase activity in the leaves can be seen in the Table 5.1. Withering by oven at 35 ˚C and by MW at 180 W retained the myrosinase activity at 16 % and 26 %, respectively. While, after a MW treatment at 900 W no myrosinase activity was detected in the leaves. Accordingly, various studies reported that microwaving is an efficient treatment for partial or complete inactivation of myrosinase in *Brassica* spp, by varying the time-temperature and power (Verkerk and Dekker, 2004; Rungapamestry et al., 2006; Fuller et al., 2007). The MW conditions and amount and type of vegetables determine largely the degree of enzyme inactivation. Moreover, thermal inactivation of myrosinase depends also on the plant source, with reported inactivation temperatures in the range from 30 to 60 ˚C (Yen and Wei, 1993; Ludikhuyze et al., 1999).

Table 5.1. Myrosinase activity (units/g DM) of withered and fermented Indian mustard leaves (n = 3)

	Withering treatment		
	Oven	**MW 180**	**MW 900**
After withering	26.5 ± 1.9	41.9 ± 4.7	n.d.
Fermentation day-1	0.043 ± 0.004	0.109 ± 0.018	n.d.
Fermentation day-3 to 7	n.d.	n.d.	n.d.

- Oven, MW 180, and MW 900 = oven at 35 °C for 2.5 h, microwave at 180 W for 4.5 min, and at 900 W for 2 min, respectively
- n.d. = not detectable
- Values are presented as means ± standard deviations.

Myrosinase activity during the fermentation

The present study shows that the remaining myrosinase activity in the oven and MW at 180 W treated samples was almost completely lost after one day of fermentation (Table 5.1). Further fermentation led to complete loss of myrosinase activity in all samples. Myrosinase activity was also not detected in the fermentation medium (data not shown). In accordance, Kim and Rhee (1993) reported a decrease of myrosinase activity from the radish tissue after three days of fermentation until less than 3%. They explained this by reduced stability due to the decrease of pH and the possible production of microbial proteolytic enzyme during fermentation. The initial pH of the fermentation medium in the present study was 4.6 and decreased after one day and during fermentation of *sayur asin* of all treatments to the range of 3.3–3.7. Moreover, the high water activity of the sayur asin may affect the myrosinase stability. A previous study reported that the inactivation rate of myrosinase in broccoli increases with the increase of water activity of the sample (Oliviero et al., 2014a)

<u>Glucosinolate concentration</u>

Glucosinolates in raw Indian mustard

Sinigrin was found to be the predominant aliphatic GS in Indian mustard accounting for more than 95% of the total GSs with a concentration of 179 ± 52 µmol/ 10 g DM (Table 5.2). Many studies on the corresponding isothiocyanate of the sinigrin, the allyl isothiocyanate, show that the isothiocyanate inhibits proliferation of human prostate and bladder cancer cells (Xiao et al., 2003; Bhattacharya et al., 2010).

Three indole GSs, i.e., 4-hydroxy-glucobrassicin, glucobrassicin, and 4-methoxy-glucobrassicin, were detected in the Indian mustard leaves in total accounting for about 5.8 µmol/ 10 g DM. In general, the profile of the main GSs is in accordance with previous studies on Indian mustard (He et al., 2003a; Font et al., 2004; Krumbein et al., 2005). However, other minor GSs, such as progoitrin, glucobrassicanapin, gluconapin, neoglucobrassicin, and gluconasturtiin, have been reported (Palmer et al., 1987; Krumbein et al., 2005). Differences in the GS concentration and profile could be due to the variations in cultivars, growing conditions, environmental factors, and the analytical methods (Palmer et al., 1987; He et al., 2003b; Krumbein et al., 2005; Martinez-Villaluenga et al., 2009; Verkerk et al., 2009).

The effect of withering methods on glucosinolates

Withering Indian mustard leaves caused a considerable reduction of total GSs ranging from about 50% by MW treatments to 70% by oven treatment (Table 5.2). Decrease of GSs during withering could be caused by thermal degradation and myrosinase hydrolysis. Previous studies reported a lower GS loss upon MW treatment on cabbage (Verkerk and Dekker, 2004; Rungapamestry et al., 2006) than the present study. The different size and shape as well as structural matrix between Indian mustard and cabbage could have led to a different loss of GSs in Indian mustard. Moreover, the damage of the cell membranes in the leaves containing active myrosinase, followed by diffusion, may have allowed myrosinase to hydrolyse the GSs, which can occur at a different rate for different vegetables.

Glucosinolates during fermentation

After the first day of fermentation, there was a marked drop of total GSs in all samples (Table 5.3). The MW at 180 W treated sample led to the greatest loss of up to 95%, followed by up to 90% for the oven treated sample, and up to 70% for the MW at 900 W treated sample, relative to the total GS concentration upon withering. Furthermore, GSs were not detectable after the third day of fermentation in the *sayur asin* previously withered in the oven and in the MW at 180 W. The withering by MW at 900 W resulted in a retention of sinigrin of 13% upon fermentation when compared to the concentration after withering (Table 5.3), while negligible amount of sinigrin was found in the fermentation medium (data not shown).

Table 5.2. Glucosinolate concentration (µmol/ 10 g DM) in Indian mustard leaves treated by three withering methods (n = 6)

Glucosinolate	Raw leaves	Withering treatment		
		Oven	MW 180	MW 900
Sinigrin	178.9 ± 51.6[c]	53.3 ± 19.0[a]	85.1 ± 8.2[b]	86.0 ± 21.2[b]
4-Hydroxy-glucobrassicin	1.1 ± 0.6[a]	1.0 ± 0.4[a] (*n*=5)	1.3 ± 0.5[a]	1.5 ± 1.3[a]
Glucobrassicin	1.4 ± 0.6[b]	0.5 ± 0.4[a] (*n*=5)	0.4 ± 0.08[a] (*n*=5)	0.5 ± 0.3[a]
4-Methoxy-glucobrassicin	3.3 ± 0.9[c]	1.1 ± 0.4[a]	2.3 ± 0.4[b]	1.9 ± 0.9[b]
Total glucosinolates	184.7	55.9	89.1	89.9

- Mean reported of duplicate analyses from 3 treatment replications.
- Oven, MW 180, and MW 900 = oven at 35 °C for 2.5 h, microwave at 180 W for 4.5 min, and at 900 W for 2 min, respectively
- Values are presented as means ± standard deviations.
- Values with superscript letters on the same row, which are different are significantly different ($P < 0.05$).

Accordingly, previous studies reported that fermentation can significantly reduce the amount of GSs in vegetables (Tolonen et al., 2002; Ciska and Pathak, 2004; Suzuki et al., 2006; Martinez-Villaluenga et al., 2009). During *sauerkraut* production, Tolonen et al. (2002) observed a very low quantity of 4-methoxy-glucobrassicin, while others reported traces or even no detectable GSs in the fermented product (Ciska and Pathak, 2004; Martinez-Villaluenga et al., 2009). Kim and Rhee (1993) also reported a 25% loss of total GSs in radish (*Raphanus sativus* L.) compared to the total GSs in raw radish on the third day of radish *kimchi* fermentation. Moreover, Suzuki et al. (2006) reported smaller quantities of the GSs in *nozawana-zuke*, a fermented product of *Brassica rapa* L. in Japan, relative to the raw *nozawana*. It could be that the cell and vacuole walls were damaged during fermentation due to the increase of osmotic pressure as a result of difference concentration of salt and water activity. Consequently, hydrolysis of GSs by active myrosinase could have occurred.

Table 5.3. Glucosinolate concentration (µmol/ 10 g DM) in Indian mustard leaves during the production of *sayur asin* (n = 6)

Glucosinolate	Withering treatment	Fermentation (days)			
		1	3	5	7
Sinigrin	Oven	4.5 ± 2.6[a]	n.d.	n.d.	n.d.
	MW 180	3.3 ± 0.7[a]	n.d.	n.d.	n.d.
	MW 900	22.6 ± 5.8[b,2]	12.3 ± 2.9 [1]	11.3 ± 1.8 [1]	11.4 ± 2.5 [1]
4-Hydroxy-glucobrassicin	Oven	0.6 ± 0.3[ab]	n.d.	n.d.	n.d.
	MW 180	0.3 ± 0.1[a]	n.d.	n.d.	n.d.
	MW 900	1.2 ± 0.7[b,2]	1.4 ± 0.5 [2]	1.7 ± 1.1 [2]	0.4 ± 0.2 [1]
Glucobrassicin	Oven	n.d.	n.d.	n.d.	n.d.
	MW 180	n.d.	n.d.	n.d.	n.d.
	MW 900	n.d.	n.d.	n.d.	n.d.
4-Methoxy-glucobrassicin	Oven	0.2 ± 0.1[ab]	n.d.	n.d.	n.d.
	MW 180	0.2 ± 0.05[a]	n.d.	n.d.	n.d.
	MW 900	0.4 ± 0.1[b]	0.2 ± 0.06	n.d.	n.d.

- Mean reported of duplicate analyses from 3 treatment replications.
- Oven, MW 180, and MW 900 = oven at 35 °C for 2.5 h, microwave at 180 W for 4.5 min, and at 900 W for 2 min, respectively n.d. = not detectable
- Values are presented as means ± standard deviations.
- Values with superscript letters on the same column and superscript number on the same row, which are different are significantly different ($P < 0.05$).

The sample that was withered by MW at 900 W does not contain active myrosinase showed a much lower reduction of GSs during fermentation. Accordingly, Sarvan et al. (2013) observed that GSs were better retained during fermentation in *sauerkraut* where myrosinase was inactivated (by blanching) prior to fermentation. However, the GSs were reduced (about 50%

reduction for sinigrin) in the pre-blanched upon 71 h fermentation samples. In the present study, about 80% of GSs were lost during the fermentation even when the myrosinase was inactivated upon withering. Therefore, it might be that during fermentation of the *sayur asin* some myrosinase-like activity was produced by bacteria that catalysed the GS hydrolysis (Shapiro et al., 1998; Oliviero et al., 2014b).

The present study shows that inactivation of myrosinase in Indian mustard by using a microwave heat treatment can lead to a partial retention of GS upon fermentation in the product. Ingestion of GS-containing products without active plant myrosinase can still result in the formation and bioavailability of bioactive breakdown products by enzymes from the gut flora, although the catalytic efficiency of the plant endogenous myrosinase is higher (Krul et al., 2002; Higdon et al., 2007; Rungapamestry et al., 2007; Traka and Mithen, 2009; Oliviero et al., 2014b).

The GSs breakdown product formation upon withering and fermentation should be also analysed to better understand the mechanisms that lead to different breakdown profiles in the final product. Among many other factors, the pH plays an important role to influence the breakdown product profile (Verkerk et al., 2009). Moreover, the activity of epithiospecifier proteins may also affects the further conversions upon enzymatic GS hydrolysis, i.e., the nitriles formation is higher when such proteins are active (Verkerk et al., 2009).

Lactic acid bacteria and colony count during sayur asin fermentation

The natural lactic acid bacteria (LAB) on the leaves were not detected after microwave withering. Therefore, a standard inoculum was added into coconut water prior to fermentation. The total LAB and total colony count in *sayur asin* during fermentation are shown in Table 5.4. Total LAB increased only until the first to third day of fermentation followed by a steady decline into 7.1–7.3 log CFU/mL. There is no difference in effect of withering methods on the total LAB in the samples. It is likely that in each sample there were similar conditions that led to similar LAB growth. A previous study on microbial ecology of *sayur asin* reported a successional development of species within the LAB group during *sayur asin* fermentation. Fermentation was initiated by *Leuconostoc mesenteroides* and *Lactobacillus confusus* and completed by *Lac. curvatus, Pediococcus pentosaceus* and *Lac. plantarum* (Puspito and Fleet, 1985).

Another study on microbial community of *fu-tsai* and *suan-tsai*, traditional fermented mustard (*Brassica juncea* Coss.) products of Taiwan, reported a high level of diversity in LAB at the different stages of fermentation, including *Enterococcus faecalis*, *Lac. alimentarius*, *Lac. brevis, Lac. plantarum, Leu. citreum*, *Leu. mesenteroides*, *Ped. pentosaceus*, *Weissella cibaria*, and others (Chao et al., 2009). The diversity of LAB was also reported during fermentation of cabbage *kimchi* (Lee, 1997) and *sauerkraut* (Plengvidhya et al., 2007).

The present study also found that total colony in *sayur asin* increased to 8.8–9.2 log CFU/mL after the first day of fermentation as compared to the ones in the withered leaves, followed by a decline into about 7.7–7.9 and 6.7–7.3 log CFU/mL after the fifth and seventh day of fermentation, respectively. Accordingly, Puspito and Fleet (1985) reported the total bacterial count is similar with the total LAB count; therefore, LAB are mainly responsible for the fermentation of *sayur asin* fermentation. Additionally, total yeast was detected at 2.6 ± 0.4 log CFU/mL at day 0 but, no yeast colonies were detected after the seventh day of fermentation (data not shown). At day 0 total *Bacillus cereus* was also detected, but uncounted due to interference of other microbes. While, at the seventh day of fermentation the total *Bacillus cereus* was not detected (data not shown).

Table 5.4. Total lactic acid bacteria and total colony count (log CFU/mL) during *sayur asin* fermentation (n = 3)

			Withering treatment					
			Oven		**MW 180**		**MW 900**	
	LAB	COL	LAB	COL	LAB	COL	LAB	COL
Raw leaves	4.4 ± 0.0	7.2 ± 0.2						
After withering			n.d.	3.4 ± 0.1^{a}	n.d.	5.7 ± 0.5^{b}	n.d.	6.4 ± 0.5^{b}
Fermentation day-1			8.9 ± 0.2^{1}	8.8 ± 0.1^{a}	9.0 ± 0.3^{1}	9.2 ± 0.1^{b}	9.1 ± 0.1^{1}	9.1 ± 0.1^{b}
Fermentation day-3			9.1 ± 0.2^{1}	9.0 ± 0.1^{a}	9.3 ± 0.1^{1}	8.9 ± 0.1^{a}	9.0 ± 0.2^{1}	9.0 ± 0.2^{a}
Fermentation day-5			8.0 ± 0.3^{1}	7.9 ± 0.4^{a}	7.9 ± 0.2^{1}	7.9 ± 0.1^{a}	7.8 ± 0.2^{1}	7.7 ± 0.2^{a}
Fermentation day-7			7.2 ± 0.2^{1}	6.7 ± 0.2^{a}	7.1 ± 0.3^{1}	6.9 ± 0.3^{ab}	7.3 ± 0.2^{1}	7.3 ± 0.2^{b}

- Oven, MW 180, and MW 900 = oven at 35 °C for 2.5 h, microwave at 180 W for 4.5 min, and at 900 W for 2 min, respectively
- LAB, COL = total lactic acid bacteria, total colony count
- n.d. = not detectable
- Values are presented as means ± standard deviations.
- Values with superscript letters or numbers on the same row, which are different are significantly different ($P < 0.05$).

Conclusions

Sinigrin is the most dominant GS in raw Indian mustard, accounting for about 95% of the total GSs with a concentration of 179 ± 52 μmol/ 10 g DM. Both withering and fermentation substantially reduced the GS concentration in the final *sayur asin* product. The myrosinase activity contributed strongly to the remaining GS in the vegetable upon fermentation. A withering treatment that fully inactivates myrosinase results in a fermented product containing substantial amounts of GS, whereas treatments with residual myrosinase activity result in products void of GS. The total lactic acid bacteria and total colony counts were not affected by the preparation methods. Lactic acid bacteria are mainly responsible for the fermentation of *sayur asin*.

The present study revealed the importance of myrosinase activity on GS concentration of Indian mustard during fermentation. This finding can contribute to design the *sayur asin* production in order to obtain a final product that has higher GS concentration plus the probiotics of a fermented product. Nevertheless, further studies are needed to identify the factors, i.e., pH, salinity, substrate, and lactic acid bacteria, and the breakdown products of the GS hydrolysis to better understand the mechanisms underlying the GS degradation during fermentation. Moreover, other withering methods can be investigated such as blanching and steaming, to better retain GSs upon *sayur asin* production.

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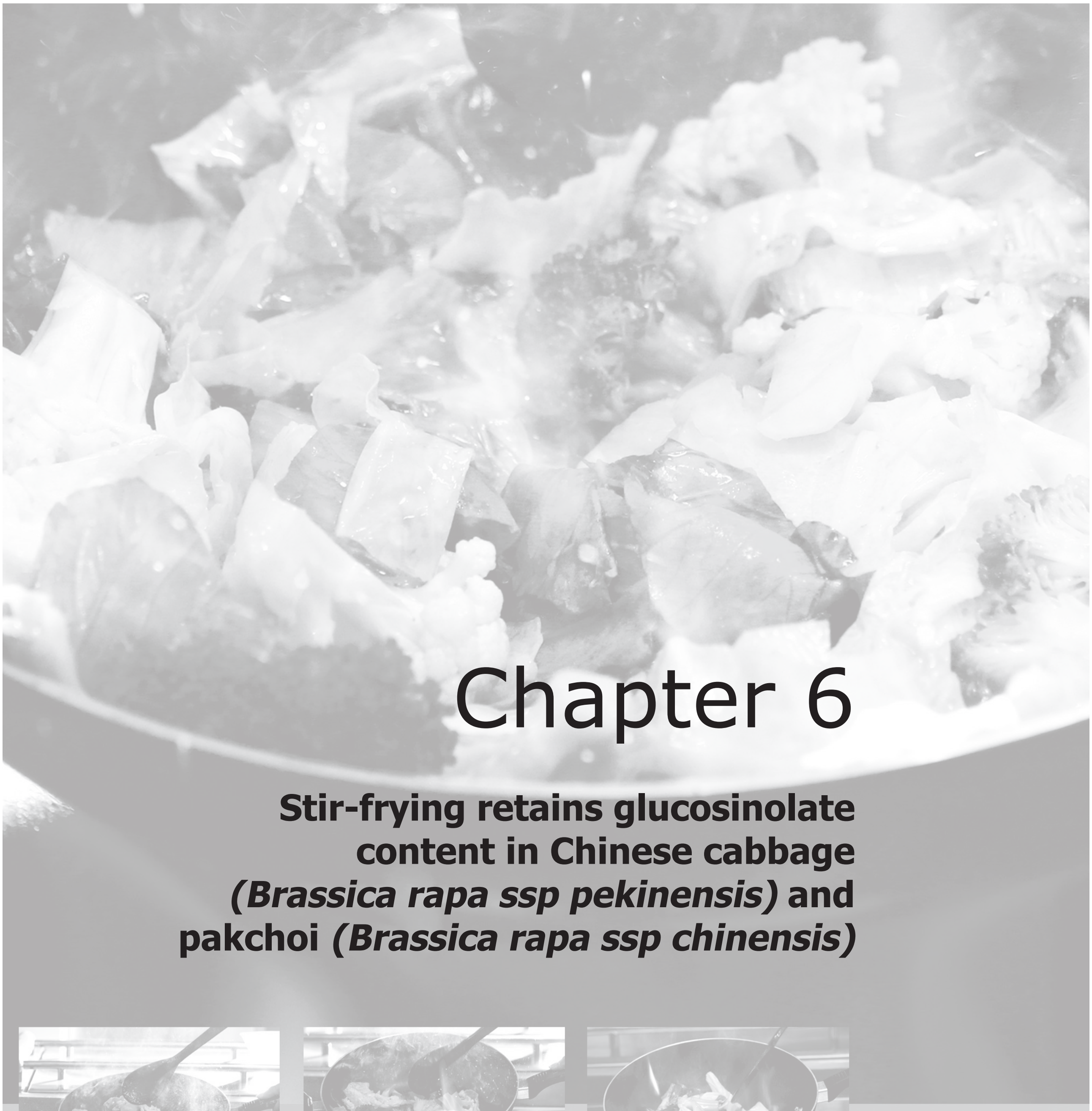

Chapter 6

Stir-frying retains glucosinolate content in Chinese cabbage *(Brassica rapa ssp pekinensis)* and pakchoi *(Brassica rapa ssp chinensis)*

Probo Y. Nugrahedi, Teresa Oliviero, Jenneke K. Heising,
Matthijs Dekker, and Ruud Verkerk

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Abstract

Stir-frying is a popular method to prepare vegetables in Southeast Asian countries that is becoming more common nowadays elsewhere in the world. Due to the short cooking time and no addition of water, stir-frying potentially can retain healthy phytochemicals that are often lost upon cooking. However, the retention of phytochemicals like glucosinolates in *Brassica* vegetables is less explored for stir-frying in comparison to other cooking methods. This study investigates the retention of glucosinolates in Chinese cabbage (*Brassica rapa* ssp *pekinensis*) and pakchoi (*Brassica rapa* ssp *chinensis*) as affected by stir-frying at various times and temperatures. Stir-frying experiments were performed at set pan temperatures ranging from 160 to 250 °C for a duration of 1 to 8 min. Result showed that aliphatic glucobrassicanapin is the most abundant glucosinolate identified in Chinese cabbage and pakchoi, contributing for 48% and 63% of the total glucosinolate content, respectively, followed by glucoiberin and gluconapin. Stir-frying retained the glucosinolate content and could even increase the extractability of the glucosinolates. The retention of glucosinolates indicates that myrosinase was inactivated, preventing the enzymatic hydrolysis of glucosinolates. Moreover, due to the absence of a separate water phase, leaching losses did not occur, in contrast to what is observed when boiling *Brassica* vegetables. No clear trends in the effects of stir-frying time and temperature were observed. The high retentions of glucosinolates during stir-frying are comparable to short time steaming and microwave processing of *Brassica* vegetables.

Keywords glucosinolate, stir-fry, Chinese cabbage, pakchoi

Introduction

Stir-frying, a popular method to prepare vegetables in Southeast Asian countries (Van Esterik, 2008), is becoming more common nowadays elsewhere in the world. Stir-frying is a quick preparation method by heat transfer from a hot pan surface to foods, using a small amount of cooking oil. *Brassica* vegetables, including Chinese cabbage (*Brassica rapa* ssp *pekinensis*) and pakchoi (*Brassica rapa* ssp *chinensis*), are commonly prepared by this technique. Various combinations of stir-frying time and temperature can be employed in which texture and colour of the product may be used as indicators to end the preparation.

Intake of *Brassica* vegetables was reported to have inverse association with the risk of certain cancers in epidemiological studies (Voorrips et al., 2000a; Voorrips et al., 2000b; Herr and Büchler, 2010). Glucosinolates (GSs), secondary metabolites present in *Brassica* vegetables, have been widely investigated to play an important role in this health promoting property. Isothiocyanates, one of GS breakdown products, have been reported to have the ability to inhibit the initiation and development of cancer (Hanschen et al., 2014).

A GS comprises of a β-thioglucoside N-hydroxysulphate with a sulphur linked β-D-glucopyranose moiety and side-chain group, which is derived mainly from methionine, tryptophan, or phenylalanine. The GS can be classified either as aliphatic, aromatic, or indole (Mithen et al., 2000; Verkerk et al., 2009). In intact plant tissue GSs are stored in compartments that are physically separated from compartments containing myrosinase enzyme (thioglucosidase EC 3.2.1.147). However, upon tissue damage, GSs can come into contact with and be hydrolysed by myrosinase, producing a range of breakdown products including isothiocyanates (Fahey et al., 2001; Hanschen et al., 2014).

Previous studies have indicated that the GS-myrosinase system is modified during thermal processing of *Brassica* vegetables due to inactivation of myrosinase, thermal breakdown of GSs and their hydrolysis products, loss of enzymatic cofactors, cell lysis and subsequent leaching of GSs and their derivatives into the cooking medium (Verkerk et al., 2009; Nugrahedi et al., 2015). However, the extent of these losses probably depends on the duration and type of heat treatment, the degree of cell lysis, and the vegetable matrix (Dekker et al., 2000; Dekker et al., 2009).

Many domestic preparation methods of *Brassica* vegetables, such as boiling, steaming, and microwave processing have been extensively studied in detail as reviewed by Nugrahedi et al. (2015). While, to our knowledge, only limited information is available on the effect of stir-frying on GS content in *Brassica* vegetables (Song and Thornalley, 2007; Rungapamestry et al., 2008; Yuan et al., 2009; Xu et al., 2014). Also, some conflicting results were reported. On one side, Song and Thornalley (2007) and Rungapamestry et al. (2008) observed the retention of total GSs and most of individual GS content in green cabbage, broccoli, Brussels sprouts, and cauliflower during stir-frying. On the other side, stir-frying of broccoli and red cabbage was reported to reduce total GSs content by 58% and 77%, respectively (Yuan et al., 2009; Xu et al., 2014). These differences might be due to specific stir-frying conditions applied and the effect of heating on the extraction efficiency of the GSs (Nugrahedi et al., 2015).

Moreover, the described studies mainly focused on the effect of one stir-frying treatment to the GS changes in *Brassica* vegetables and compared the results with other preparation methods. Very little information is available on the mechanism of GS changes during stir-frying as affected by various times and temperatures. Different time and temperature combinations of stir-frying are hypothesized to affect the GS content at various extents. Therefore, the present study aims to investigate the stability of GSs in two *Brassica rapa* vegetables, i.e., Chinese cabbage and pakchoi, as affected by stir-frying at various times and temperatures.

Materials and Method

Sample preparation

Chinese cabbage and pakchoi were purchased in January 2014 from a local greengrocer (Wageningen, the Netherlands). Dirt and damaged leaves were removed. The stem was cut off and the vegetables were divided into equal portions of 150 g. Each portion contains leaves from different parts of the vegetable. These were chopped in 1–2 cm strips followed by either stir-frying or freezing in liquid nitrogen immediately.

Stir-frying

Each sample was stir-fried for 1, 2, 4, or 8 min using an electrical frying pan (PZ-2964, Tristar Europe BV) set at 160, 200, 225 or 250 °C. Each preparation was performed in duplicate. Ten mL of sunflower oil (Cap D'or) was poured into the pre-heated pan and heated to the set temperature. Then, 150 g vegetables were added and stirred continuously. The temperature of the pan during stir-frying was regularly monitored (IR thermometer, scan-356, tservice). Upon completion the stir-fried vegetables were collected and frozen immediately using liquid nitrogen. Subsequently, the samples were freeze-dried (Christ Alpha 1-4 LD plus) and grinded. The samples were kept frozen at -22 °C for further analysis.

Glucosinolate analysis

Duplicate analysis of GS was performed involving extraction and desulphation steps as described by Verkerk et al. (2001) with minor modifications. Briefly, 0.2 g of the freeze dried sample was added with 2.4 mL hot methanol 70% and 3 mM glucotropaeolin solution as internal standard. Subsequently, each sample was incubated in a water bath for 20 min at 75 °C, and mixed every 5 min. The sample was then centrifuged for 10 min at 2500 rpm. The supernatant was collected and the pellet was re-extracted twice with 2 mL of hot methanol following similar procedure. All supernatants were collected and used for desulphation.

The extracted GSs were desulphated on-column, involving sulphatase enzyme, for overnight at ambient temperature. Subsequently, the desulpho-GSs were analysed by HPLC equipped with C-18 column (Merck) at a flow rate of 1 mL/min and the injection volume of 20 μL. Elution of desulpho-GSs from the HPLC column was performed by a gradient of milli-Q water and acetonitrile for 25 min of running time and subsequent measured at a wavelength of 229 nm. Each GS was identified by its UV spectra and quantified by using relative response factor relative to the internal standard glucotropaeolin. The total GS content was calculated as the sum of all identified GSs.

Results and Discussion

Stir-frying temperature

The temperature of the pan varied with time and location on the pan surface during stir-frying. Although different temperature settings were used, resulting in very different measured temperatures of the pan just before the addition of the vegetables (measured pan surface temperatures ranging from 165–251 °C), the temperature of the pan decreased quickly upon the addition of the vegetables followed by a slow increase during stir-frying. Due to this phenomenon the resulting average temperatures during stir-frying varied considerably less (measured pan surface temperatures ranging from 152–198 °C) than the temperature prior to stir-frying.

Glucosinolates in Chinese cabbage and pakchoi

Three aliphatic glucosinolates (GSs), i.e., glucoiberin, gluconapin, glucobrassicanapin, and three indole GSs, i.e., glucobrassicin, 4-methoxy-glucobrassicin, and neoglucobrassicin, were identified in both raw vegetables (Table 6.1). The aliphatic glucobrassicanapin is the most abundant GS in Chinese cabbage and pakchoi, accounting for about 48% and 63% of the total GSs, respectively, followed by glucoiberin for Chinese cabbage (31% of the total

GSs) and gluconapin for pakchoi (16% of the total GSs). Meanwhile, the total content of indole GSs contributes about 20% and 10% of the total GSs in Chinese cabbage and pakchoi, respectively.

The total GS concentration in these two *Brassica rapa* vegetables is comparable with that reported in a previous studies but there are differences in the profile of major GSs (Lewis and Fenwick, 1988; Chen et al., 2008; Lee et al., 2014). Lewis and Fenwick (1988) reported the main GSs to be progoitrin, gluconapin, glucoalyssin, and glucobrassicanapin. In other studies, glucobrassicin and gluconasturtiin were also reported as major GSs in Chinese cabbage and pakchoi (Chen et al., 2008; Lee et al., 2014). Many agronomic factors, such as vegetable variety and environment, and method of analysis can influence the GS profile and concentration differences (Lewis and Fenwick, 1988; Kang et al., 2006; Hanson et al., 2009).

Table 6.1. Glucosinolates (µmol/g DW) in raw Chinese cabbage and pakchoi

	Chinese cabbage	**Pakchoi**
Glucoiberin	0.95 ± 0.08	0.17 ± 0.09
Gluconapin	0.07 ± 0.01	0.37 ± 0.05
Glucobrassicanapin	1.47 ± 0.16	1.46 ± 0.14
Glucobrassicin	0.24 ± 0.07	0.09 ± 0.02
4-Methoxy-glucobrassicin	0.29 ± 0.01	0.13 ± 0.02
Neoglucobrassicin	0.04 ± 0.01	0.07 ± 0.02
Total glucosinolates	3.07 ± 0.17	2.29 ± 0.16

Values are presented as mean ± standard deviation (n = 4)

Effect of stir-frying

The total GS content of Chinese cabbage after the first minute of stir-frying increased by 30–100% compared to the content measured in the raw vegetable (Figure 6.1). This trend is mainly caused by the aliphatic glucobrassicanapin and glucoiberin as the major GSs. Total indole GSs only slightly increase after initial stir-frying (Figure 6.1). Accordingly, different preparation methods were reported to increase GS content after preparation, such as after microwave processing of red cabbage (Verkerk et al., 2001), boiling of cauliflower (D'Antuono et al., 2007), and steaming of broccoli (Gliszczyñska-świglo et al., 2006; Miglio et al., 2008). It is likely that the heat treatment caused an increase in the extractability of GSs from the sample matrix during the analysis.

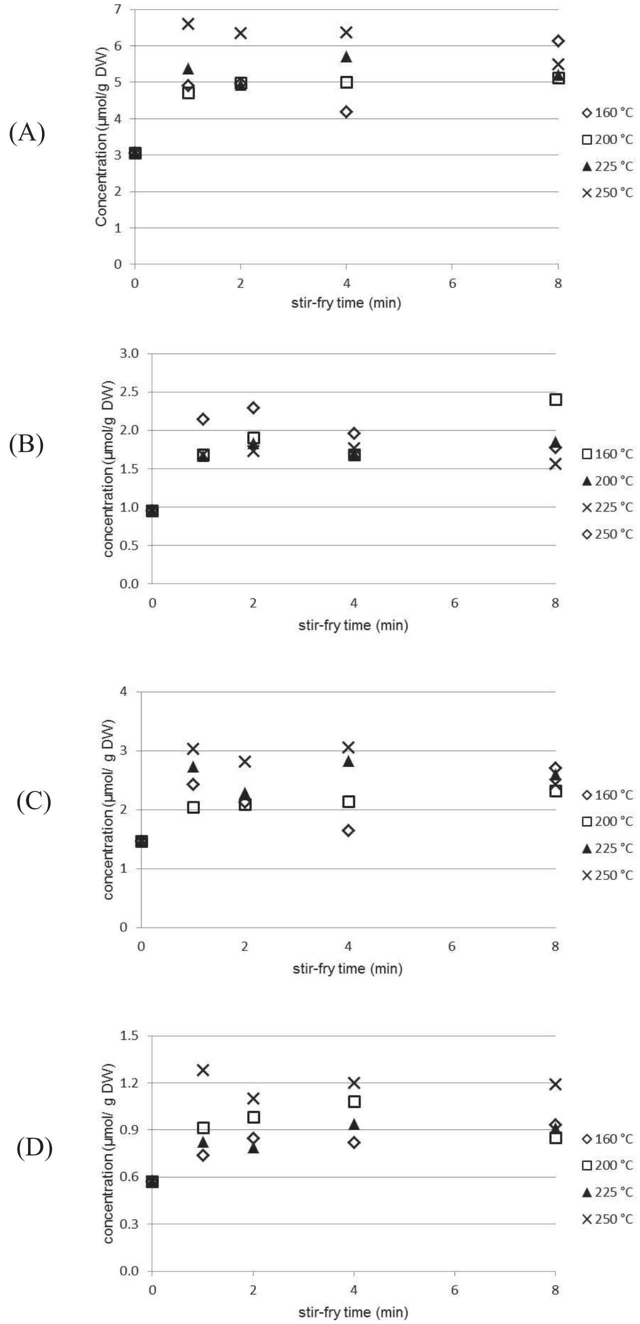

Figure 6.1. Total glucosinolates (A), glucoiberin (B), glucobrassicanapin (C), and total indole (D) glucosinolates (µmol/g DW) in Chinese cabbage during stir-frying (n=4). The legend shows the set stir-frying temperatures of the pan.

No clear increase in extracted GSs is observed for pakchoi, in contrast to Chinese cabbage (Figure 6.2). Only after stir-frying at 160 °C for 4 min a substantial increase of total GSs can be observed compared to the raw pakchoi. This increasing trend is mainly caused by gluconapin and glucobrassicanapin.

The plant matrix in which the GSs are located, and its composition, could play an important role in the rate of GS degradation (Dekker et al., 2009). Compared to Chinese cabbage, physically pakchoi has a more leafy structure and its leaves are thinner, so they are expected to heat up faster and to release more moisture during stir-frying. This moisture may contain GSs that are then exposed to the high temperature of the pan surface, resulting in a fast breakdown, balancing the increased extractability in the vegetable tissue.

Heat is transferred mostly by conduction from the hot surface of the pan or wok through a thin layer of hot oil. So, the surface temperature of vegetable rises rapidly and a proportion of water is vaporised (Fellows, 2009). Consequently, this rapid heating leads to myrosinase inactivation. Rungapamestry et al. (2008) reported an 83% reduction in myrosinase activity in broccoli florets upon stir-frying for 4 minutes. In the present study, since the high frying temperatures were applied and the fact that thin strips of leaves were used, most of myrosinase from both vegetables is expected to be inactivated at the beginning of stir-frying, resulting in a negligible influence on the GS hydrolytic degradation.

The effect of various times and temperatures during stir-frying on the GS content cannot be clearly seen in the present study. For both vegetables, the total, aliphatic, and indole GS contents are relatively stable during stir-frying. During 8 min of stir-frying the GS contents in Chinese cabbage slightly increase, as compared to the changes of pakchoi. Moreover, higher set pan temperatures employed in the present study did not affect to the rate of GS changes.

The few studies performed on stir-frying of *Brassica* vegetables show considerable variation in the retention of GSs. Rungapamestry et al. (2008) demonstrated a substantial retention of total aliphatic, indole, and aromatic GSs in broccoli during stir-frying, even more than 95% as compared to the raw broccoli. Moreover, total GS content of stir-fried broccoli that was formerly blanched-and-frozen also showed no significant difference from the blanched-and-frozen broccoli (Rungapamestry et al., 2008). On the contrary, Yuan et al. (2009) observed that stir-frying of broccoli reduced the amount of the aliphatic and indole GSs by about 55% and 67%, respectively. Most recently, Xu et al. (2014) reported that stir-frying reduced 77% of total GSs in red cabbage. These conflicting results might be due to specific stir-frying conditions applied and the different type of *Brassica* vegetable.

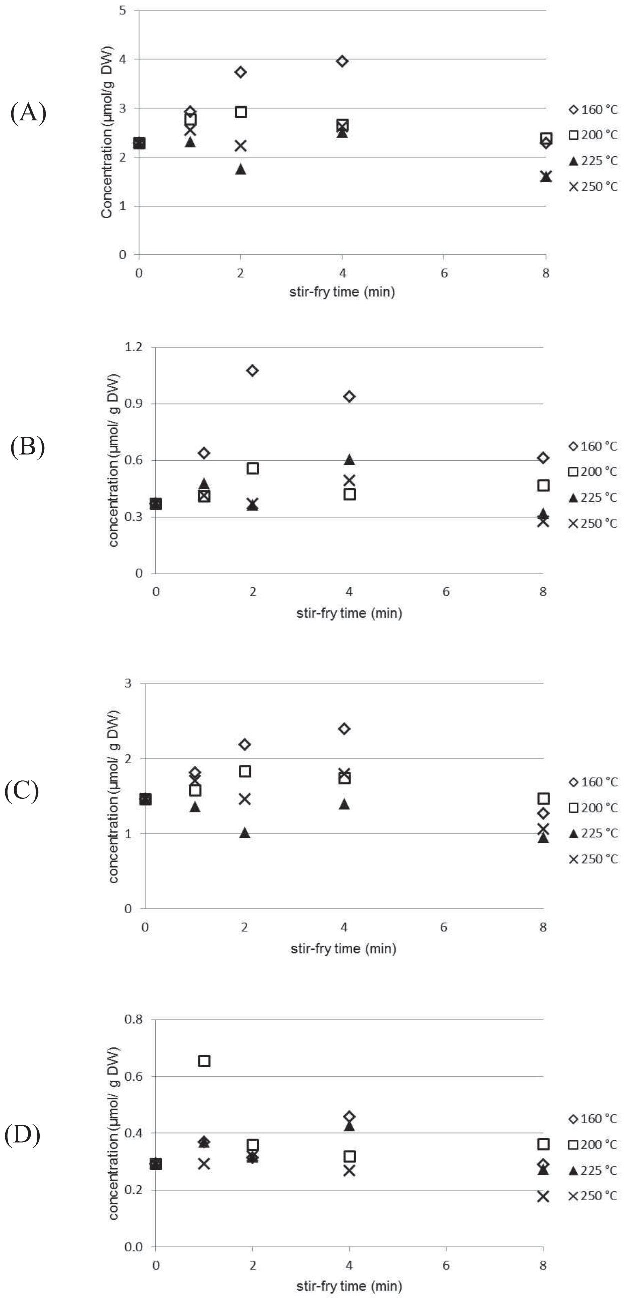

Figure 6.2. Total glucosinolates (A), gluconapin (B), glucobrassicanapin (C), and total indole (D) glucosinolates (µmol/g DW) in pakchoi during stir-frying (n=4). The legend shows the set stir-frying temperatures of the pan.

Rungapamestry et al. (2008) and Song and Thornalley (2007) employed stir-frying at lower temperature and the cutting size of the vegetables was larger than other authors (Yuan et al., 2009; Xu et al., 2014). In addition, different types of cooking oil were also found to influence to the loss of GS content (Moreno et al., 2007). Moreover, the loss of total GSs in broccoli by 84% was also reported when deep-frying was applied apparently due to the intense thermal degradation (Miglio et al., 2008).

The lack of a clear effect of the different applied temperatures on GS levels in the present study may be explained by the fact that at the beginning of stir-frying, the temperature of the main part of the vegetable tissue will not exceed about 100 °C since the tissue still contains most of its moisture. Only by increasing the stir-frying time enough water will evaporate to allow the vegetable temperature to rise further and to see a clear temperature effect. Moreover, the retention of GS can be explained by the lack of water addition during cooking. Leaching of GS is the main factor that contributes to the reduction of GS contents during boiling and blanching of *Brassica* vegetables. The main differences observed in our study compared to other published studies is the duration of the stir-frying time and the absence of a water phase, which was added after the initial stir-frying in some of the studies.

In a review on the mechanisms underlying GS changes during preparation, Nugrahedi et al. (2015) have proposed that a low degree of leaching, thermal degradation, and enzymatic hydrolysis can be expected to occur during stir-frying of *Brassica* vegetables. In the present study, at the applied time/temperature conditions, these mechanisms were indeed found to have a minimal effect, in fact the increased extractability of the GSs during stir-frying is the main responsible mechanism observed, especially in Chinese cabbage.

Conclusions

The aliphatic GS glucobrassicanapin is the most abundant GS identified in raw Chinese cabbage (*Brassica rapa* ssp. *pekinensis*) and pakchoi (*Brassica rapa* ssp. *chinensis*) contributing for 48% and 63% of total GSs, respectively. Stir-frying retains the GS content in both vegetables, in fact it could even increase the extractability of GSs, particularly in Chinese cabbage. Furthermore, there is no clear effect of different time and temperature combinations of stir-frying on the changes of GSs. Minimum leaching, low degrees of enzymatic hydrolysis and thermal degradation of GSs are expected to occur during the stir-frying treatment of these two *Brassica rapa* species. This study demonstrates that next to steaming and microwave processing, stir-frying also can retain the GS content in *Brassica* vegetables.

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Chapter 7

General Discussion



Introduction

Brassica vegetables are popular vegetables in the diets of many people globally. These are commonly prepared into various dishes prior to consumption. Processing and preparation of *Brassica* vegetables can alter the quality attributes including the content of alleged health promoting glucosinolates (GSs). Consequently, this can have a significant influence on the final intake of GSs. Therefore, the effects of processing and preparation of *Brassica* vegetables should be taken into account in order to make accurate estimates of dietary intake of these GSs (Dekker et al., 2000).

The general objective of this thesis was to investigate the GS content of *Brassica* vegetables upon consumption by considering the various steps in the production chain and to investigate whether it is possible to optimize the GS content. More specifically, the effects of (Indonesian) preparation methods on the GS content of *Brassica* vegetables were studied by applying a mechanistic perspective that underlies the GS changes during preparation. The work focused on the following research questions:

1. How do food handlers of food service establishments and households (in Indonesia) prepare *Brassica* vegetables and what is their perception on health effects upon the preparation method and *Brassica* type?
2. How does time-temperature of *Brassica* vegetable preparation, particularly steaming and stir-frying, affect the GS content?
3. What is the effect of myrosinase activity on GS content during fermentation of *Brassica* vegetables?
4. What is the relative importance of underlying mechanisms in the preparation method affecting the changes of GS content?

Main findings

Processing is one of the major factors affecting changes of GS content, mainly due to leaching and thermal degradation, along the production chain of *Brassica* vegetables. Previous publications on the effect of various processing and preparation methods on the GS content in *Brassica* vegetables were reviewed in Chapter 2. The changes of GSs were also analysed by discussing the relevant mechanisms underlying each processing method. Boiling and blanching generally reduce the GS content significantly due to cell lysis, diffusion, thermal degradation, and leaching into processing water. Meanwhile, steaming and microwave processing retain or even increase the total accessible GS content because of a low impact of thermal degradation and leaching, and an apparent increase of the GS extractability in the plant matrix during analysis. Stir-frying slightly reduced the GS content; compared to boiling and blanching the losses were only minor. For individual GS, changes are more complicated to explain because they appear to have different properties in each type of *Brassica* vegetable and different responses to each preparation method and condition.

To include a local perspective on various *Brassica* vegetable preparation methods, Chapter 3 describes the preparation practices of food handlers in food service establishments and households in Semarang city, Indonesia and their perception of health benefits of these vegetables and preparation methods. Boiling and stir-frying are the most frequently applied for preparing *Brassica* vegetables. White cabbage and choysum are the most frequently prepared vegetables compared to other *Brassica* vegetables. These vegetables are also prepared to more diverse dishes. In terms of perception of health, broccoli is perceived by the respondents as the healthiest *Brassica*. Steaming and boiling are perceived to have the highest health beneficial effects according to the food service establishment respondents. Meanwhile, for the household respondents boiling and stir-frying are perceived as the most health beneficial preparation methods.

Studies on GS content as affected by three preparation methods commonly employed to produce local food products, namely steamed cabbage roll (as part of *siomay* dish), fermented Indian mustard (*sayur asin*), and stir-fried vegetable (*tumis* or *ca*), are presented in Chapter 4 to Chapter 6. Previous studies reported that steaming can retain the GS content in *Brassica* vegetables. In Chapter 4 a short blanching of the leaf layers of white cabbage followed by a long-term steaming of cabbage roll can increase the total accessible GSs after 10 min of steaming. Subsequently, a consistent decline was observed. This behaviour is contributed mainly to the aliphatic GSs, particularly glucoraphanin and glucobrassicin, rather than to the indolyls. Moreover, steaming for up to 10 min could also retain colour and texture of cabbage roll as important quality attributes. The mechanisms underlying long-term steaming were identified to explain the behaviour of GS content by using a mathematical modelling approach. These include cell lysis, enzymatic hydrolysis, myrosinase inactivation, increase in extractability of GSs, GS thermal breakdown, steam condensation on the rolls, GS leaching to the condense water on/in the rolls, and condense water containing GSs dripping from the rolls.

Among other preparation methods on *Brassica* vegetables, the underlying mechanisms of fermentation affecting the GS changes are less explored. Chapter 5 reports the GS content in Indian mustard (*Brassica juncea*) during the fermentation in *sayur asin* production and how the activity of myrosinase affects the GS content during fermentation. Sinigrin is the most dominant GS in the raw Indian mustard, accounting for about 95% of the total GSs. Withering the leaves by oven at 35 ˚C and by microwave for 4.5 minutes at 180 W retained the myrosinase activity at 16 % and 26 %, respectively. After a microwave treatment for 2 minutes at 900 W no myrosinase activity was detected in the leaves. Withering and fermentation substantially reduced the GS content when active myrosinase is present. During fermentation, the reduction was affected by the levels of myrosinase activity from the withered leaves. The *sayur asin* of fermentation day 7, which contained inactivated myrosinase, retained the GS sinigrin at about 11.4 µmol/ g DM, whereas treatments with residual myrosinase activity resulted in products void of GS. It was also found that lactic acid bacteria are mainly responsible for the fermentation of *sayur asin*.

Stir-frying is one of the popular methods to prepare vegetables in Indonesia. Chapter 6 presents the effect of stir-frying on the GSs of Chinese cabbage (*Brassica rapa* ssp *pekinensis*) and pakchoi (*Brassica rapa* ssp *chinensis*), by varying cooking conditions, namely time and temperature. Aliphatic glucobrassicanapin was the most abundant GS identified in both *Brassica rapa* species, followed by glucoiberin and gluconapin. Stir-frying was shown to retain the GS content and even could increase the extractability of the GS content, particularly for Chinese cabbage. There was no clear effect of time and temperature of stir-frying on the GS changes. Due to the absence of a separate water phase, leaching losses did not occur.

Methodological considerations

Literature review

Several papers briefly reviewed the effect of processing on GS content in *Brassica* vegetables (e.g., Mithen et al., 2000; Rungapamestry et al., 2007; Cartea and Velasco, 2008). However, variability in processing conditions and analytical methods complicate the comparison of GS content from various processing methods (Rungapamestry et al., 2007). Hence, the present study thoroughly reviewed the recent published papers by employing the underlying mechanisms of each preparation method that are considered critical to affect the changes of GSs.

Literature research was conducted to obtain relevant data on the effects of processing on the GS content in *Brassica* vegetables. Various expressions for the unit of GS content were reported among studies. Therefore, to allow for comparison of the effects of processes across all studies, concentrations of GSs were converted to a percentage. If the study did not report either total, aliphatic, or indolyl GS content, then calculation was employed by grouping and summing of the identified GSs in the study. Calculation on total aliphatic, indolyl, and aromatic GSs was based on the published mean value of individual GSs belonging to the group, while the standard deviation or standard error of the mean were not included. Therefore, the percentage of GS retention in this study contains variability due to error propagation. However, these data can still be used to make a clear comparison within preparation methods, *Brassica* types, and between literature sources. This error is considered to be minor compared to the differences observed between these methods, types, and studies. Moreover, these data can be used to indicate to what extent the underlying mechanisms from each preparation method affect GS changes.

In order to reveal the underlying mechanisms involved, all details of preparation conditions described in the studies, e.g., time, temperature, *Brassica* size or weight, and water to vegetable ratio, were considered and discussed in the way they could have an influence on the mechanisms affecting the GS changes. Therefore, the conditions described in the review are varied between the different preparation methods. These differences indicate that underlying mechanisms affecting GS changes in each preparation method are specific. Eventually, by putting these altogether, i.e., the percentage of GS retention, the preparation conditions, and the mechanisms involved, the relative importance of the underlying mechanisms affecting GS changes due to different preparation methods were defined. These defined mechanisms were important to set the independent variables in the next studies on the effects of (Indonesian) preparation methods on the GS content. Moreover, these mechanisms could be used as foundation to formulate the model.

By including the mechanisms of changes in GS content, the review was not just about reporting the previously measured effects of processing. This approach enables to view the processing effects in a broader perspective and to define more concretely in which ways processing steps should be investigated and analysed in the experimental part of the thesis.

Survey study

In order to obtain the information on how *Brassica* vegetables are commonly prepared, a survey was conducted in Semarang city, the capital of Central Java province, Indonesia, on two groups of food handlers, i.e., food service establishments and households. Since the food handlers prepare the foods to fulfill the needs of consumers (including the needs of family members), their motivation on why preparing certain foods and employing certain methods were not investigated. Nevertheless, both groups are the vital actors that prepare the foods for daily consumption of the society. Hence, the survey focused on the preparation methods commonly employed on *Brassica* vegetables. Moreover, since the vegetables intake associates with health promotion and processing can degrade the health promoting compounds in the vegetable, the health perception of food handlers on *Brassica* types and on the effect of preparation methods was also studied.

The participating food service establishments are mainly located at or nearby the city center, campuses, main streets, or dense settlements. For the households, respondents were from four areas spread within the city (Figure 7.1). This survey was not intended to fully represent the population of food handlers of both groups in Semarang city. However, the data were coming from 123 and 477 respondents of food service establishments and households, respectively and could give an insight on the implication of the preparation methods of *Brassica* vegetables on the GS content. Demographic factors of the respondents, such as age, gender, and education level, as well as type and scale of the food service establishment may influence the variability of information in the survey. Consequently, the results of the survey only show a general overview of the preparation methods commonly applied on *Brassica* vegetable and the health perception by these two groups regardless of their motives and other specific factors.

Figure 7.1. The location of the household survey

For some questions different types of answering categories were used for the food service establishments and the households, i.e., ranking and multiple choice types, respectively. This is due to the fact that food service establishments mostly prepare fixed menu or dishes routinely while households can prepare different dishes every day. The ranking type was easily understood by the food service establishments when giving the options to rank. On the contrary, this type was difficult to be applied for the households; hence, the multiple choice type is more preferred. However, due to this difference, the results from both groups cannot be compared for these items.

Experimental design

Brassica vegetables were prepared in the laboratories to produce cabbage roll, stir-fried product, and *sayur asin* according to the local practices with some modifications. For *sayur asin* production, withering of *Brassica juncea* was performed by oven instead of by conventional sun drying due to the different climate between Indonesia and The Netherlands. The differences in atmospheric condition, such as temperature and relative humidity, may have affected the characteristics of the withered leaves. Consequently, the result of withering could not reflect the real preparation of *sayur asin*. However, withering by oven offers more advantage than conventional sun drying, namely the temperature and the cleanness of the air during withering can be controlled.

Microwave treatment was applied for withering the leaves as well as to achieve the (partial) inactivation of myrosinase from the raw vegetable. Enzyme inactivation by microwave treatment appears to be more effective than by conventional heating because the heat treatment can be set by controlling the microwave power. Therefore, different levels of myrosinase activity could be obtained by setting the microwave power differently. Moreover, this treatment offers an advantage that during microwaving leakage of GSs is limited and higher retention of GSs in the vegetable can be expected. The temperature rise in the food depends on the duration of heating, the location in the food, convective heat transfer at the surface, and the extent of evaporation of water in the food and at its surface (Verkerk and Dekker, 2004).

For the stir-frying experiments, an electrical frying pan was used in order to set and control the temperature during frying. However, the temperature profiles of the experiment can be different from the real practices. In real application, stir-frying was performed by using a frying pan or wok heated on fire produced by either a high pressure burner (mainly at food service establishments) or stove.

In this study, all productions were done in batches of pooled raw materials to ensure the homogeneity of the population and the proper sampling prior to analysis. However, due to the sample size, long-term steaming and stir-frying had to be performed in two and three consecutive days, respectively. These practices could lead to less homogeneity of the population probably resulting in less precise results.

Mathematical modelling

Mathematical modelling was employed as a tool to describe the GS glucoraphanin changes in cabbage roll during steaming. Different mechanisms taking place during steaming were identified. To build the model, the mechanisms were modelled separately and then linked together into one steaming model. In order to formulate the mathematical equations of each mechanism, some assumptions were made. For instance, the observed increased in GS extractability during long term steaming, which was not yet included in the existing models (Sarvan et al., 2012), was assumed to be caused by the fact that a part of the GSs is located in a fraction of the vegetable tissue that is not available for the extraction procedure. During steaming, steam will condensate on the cabbage rolls and part of this condensate will be absorbed by the rolls and part will drip back into the water beneath the vegetables. The measured GS concentration in the cabbage rolls is the sum of the measurable amount of GSs in the intact vegetable cells plus the GSs in the lysed vegetable cells and in the condensate in/on the rolls divided by the total mass of the rolls. This additional mechanism adds additional parameters to the model description. In order to reduce the number of parameters (principle of parsimony) (Van Boekel, 2009), one lysis rate was assumed for all cells (for cells with the extractable and cells with the non-extractable GSs). This might be an oversimplification, but the experimental data do not allow the estimation of multiple lysis parameters for different tissue types. Additionally, the GS changes due to enzymatic myrosinase hydrolysis and myrosinase inactivation were assumed to be negligible due to heat treatment during blanching prior to long-term steaming. However, myrosinase may not have been fully inactivated after 3 minutes of water blanching, as different processing time can lead to different level of inactivation (e.g., as reported during steaming by Rungapamestry et al., 2006; Verkerk et al., 2010).

Discussion and interpretation of the results

Based on the published studies on the effect of processing and domestic preparation on GS content in *Brassica* vegetables Rungapamestry et al. (2007) indicated complexities to compare and interpret data directly, due to variability of processing conditions and analytical methods. Moreover, as compared to industrial processing, domestic preparation is more complicated to control (van Boekel et al., 2010). This study showed the benefit of a mechanistic approach to discuss the GS changes as affected by domestic preparation methods. By using this approach, the relative importance of underlying mechanisms affecting GS changes of each preparation method was identified. For instance, cell lysis, diffusion in tissue, and myrosinase inactivation were identified as the main mechanisms in all preparation methods involving heat. Leaching was also identified as the main mechanism affecting GS changes in boiling but this was not the case for stir-frying. For steaming, blanching, and microwave processing, leaching could be also part of the main mechanisms but depending on the conditions of the preparation, such as the additional water and the ratio of the additional water to the vegetable. Meanwhile, thermal degradation of GS was one of the main mechanisms involved in stir-frying but for other preparation involving heat, this could play an important role depending on the conditions, such as the method (hot vs. cold start boiling), temperature, and the size of the vegetable.

These mechanisms can also be applied to indicate the cause of contradictory results between publications studying similar preparation method. For example, the contradictory results reported on the effects of microwave processing (Table 2.5, Chapter 2) were mainly due to the different levels of cell lysis, diffusion, thermal degradation of GS, myrosinase inactivation, and increased extractability. Leaching can also affect GS loss when considerable amounts of water are added. Moreover, the conditions of the preparation, which influence to what extent underlying mechanisms affect GS changes, were identified for the purpose of optimisation of domestic preparation. In the case of microwave processing, processing time and power output (and the amount of additional water, if any) must be carefully controlled to obtained the highest possible GS content. The relative importance of underlying mechanisms was identified after putting altogether the reported conditions of the preparation, the calculated GS retention, and the possible underlying mechanisms affecting GS content during preparation.

This mechanistic approach is illustrated in the following process of boiling or water blanching. The mechanisms were identified starting from the heat of water that is transferred into the vegetable tissue leading to cell lysis. Subsequently, GSs and the myrosinase will diffuse through the lysed tissue and partly leach into the cooking water. This brings myrosinase in contact with GSs, hence, enzymatic breakdown of GSs can occur both in the damaged tissue and in the cooking water. At the same time, denaturation of myrosinase can occur due to the high temperature. Depending on the rate of temperature rise of the vegetable tissue and the stability of the specific myrosinase, enzymatic breakdown of GSs is usually expected to be limited. Thermal degradation of GSs occurs at boiling temperature. Leaching is the major factor of GS loss during boiling and water blanching (Chapter 2). However, contradictory results, e.g., the retention of GS content after boiling or blanching, were also reported; as also found in the present study that the GS content was relatively stable when water blanching of leaf layers of white cabbage for three minute was employed (Chapter 4). This short heat treatment does not result in intensive GS loss due to enzymatic hydrolysis, thermal breakdown, and leaching. It is likely that most of the hydrolytic enzyme myrosinase had been inactivated and there might be an increase of extractability during analysis. Thus, preparation time and temperature, the ratio of vegetable to water, the boiling method, and the type and geometrical shape of the vegetable tissues are the factors strongly affecting the behaviour of GS content during boiling and water blanching (Dekker et al., 2000).

Another benefit of using this mechanistic approach is that the GS content as well as all factors/conditions involved in the preparation are described, predicted, and optimised quantitatively by employing kinetic modelling. Kinetic modelling is a tool in understanding what is going on because proposed mechanisms need to be confronted with experiments. Compared to empirical models, a mechanistic model has the advantage of giving insight in the various sub-processes and allow for more flexibility and accuracy in use outside the studied experimental settings (Van Boekel, 2009; Sarvan et al., 2012). In the present study, the mathematical model for glucoraphanin in the cabbage rolls fitted the observed concentration profile during steaming (Chapter 4). This implies that the mathematical model can be used to predict the changes of GS content and the underlying mechanisms are well defined to formulate the model. However, further optimisation on the conditions of steaming to achieve the highest possible GS content in the product should be performed, e.g., by studying the effects of the size of the cabbage roll and the ratio of the water, steam, and rolls, on the retention of GS content and myrosinase activity.

The cabbage roll is prepared by a short blanching of the leaf layers of cabbage followed by rolling and (long-term) steaming. By the short blanching time the loss due to cell lysis and leaching is minimised; but still a low degree of enzymatic hydrolysis of GSs can be expected. Until 10 min of steaming total GSs of cabbage roll increased, then a consistent decline was observed until the rest of 180 min of steaming (Chapter 4). At short heat treatments a higher extractability of GSs is attributed in the tissues. The similar behaviour on the increase of GS content after preparation was also reported in the chopped cabbage (Verkerk et al., 2001), microwaved cabbage (Verkerk and Dekker, 2004), and stir-fried Chinese cabbage (Chapter 6). Most of the hydrolytic myrosinase was expected to be inactivated by the blanching step prior to steaming. The decrease in GS content will be mainly caused by thermal degradation.

Leaching into the condensed steam is expected at low level followed by drip loss. Thus, the duration of steaming is the critical factor affecting GS content in the case of cabbage roll.

Among other methods commonly applied to prepare *Brassica* vegetables, fermentation may have specific underlying mechanisms affecting the changes of GS content and myrosinase activity, such as the reactions with salt (ions), sugars and other compounds in the substrate, and/or myrosinase-like of lactic acid bacteria. Some conditions in fermentation will affect the changes due to these mechanisms, e.g., microbiological profiles, concentration of salt and sugar, pH, type of medium, ratio of water (medium) to vegetable, and time-temperature. However, little information is available on fermentation studies on GSs in *Brassica* vegetables. GS hydrolysis by myrosinase activity is one of these mechanisms strongly influencing the changes of GS during fermentation of *Brassica* vegetable. Therefore, the effect of myrosinase activity was studied during fermentation of Indian mustard (*Brassica juncea*) in the production of *sayur asin*. GSs in *sayur asin* are not detectable after the third day of fermentation, when active myrosinase is still present in the withered leaves prior to fermentation. The sample that was withered by microwave at high power did not contain active myrosinase and consequently, showed a much lower reduction of GSs during fermentation (Chapter 5). This study showed that the retention of GS content in *sayur asin* was achieved by inactivating myrosinase from the vegetable prior to fermentation. However, the level of GS retention was only about 13% after seven days of fermentation. This indicates that mechanisms other than myrosinase-catalytic hydrolysis play a significant role to degrade GS content during preparation. As the breakdown of GS can also occur in the intestine due myrosinase-like activity of the gut flora (Oliviero et al., 2014b), the microbiological profile in the fermentation may also contribute to the GS reduction in the present study. Hence, further study on the effect of fermentation by using certain microorganism on GS content in *sayur asin* is necessary. Sarvan *et al.* (2013) reported that fermentation of blanched cabbage by *Lactobacillus paracasei* LMG P22043 produces high content of GSs in sauerkraut in combination with a high count of live probiotic bacterial cells.

Moreover, the withering by microwave treatment also reduced considerably the concentration of GSs as compared to the GS content in the raw *Brassica*. Hence, further study can be performed to optimise this withering treatment, e.g., by optimising the combination of microwave power and vegetable weight, in order to have a higher GS retention in the sample prior to fermentation. In the case of microwaving red cabbage, Verkerk and Dekker (2004) reported that various microwave powers influence the GS content and at high power the GS content can be best retained. However, microwave processing is not a familiar method to prepare vegetables in Indonesia. Therefore, another alternative for withering as well as inactivating myrosinase should be investigated, e.g., by blanching (Sarvan et al., 2013).

Time and temperature during heating are the vital factors affecting the GS content of *Brassica* vegetables. Stir-frying, one of the most popular methods to prepare vegetables, was performed at various times and temperatures (the set pan temperatures ranging from 160 to 250 °C) to study their impacts on GS content. The retention the GS content in Chinese cabbage and pakchoi after stir-frying (Chapter 6) indicates the low degrees of cell lysis and diffusion, leaching, thermal degradation, and enzymatic hydrolysis mechanisms. It is likely that the actual product temperature was much lower than the set pan temperature, thereby limiting thermal degradation. During stir-frying the temperature of the main part of the vegetable tissue will be about 100 °C since the tissue will still contain most of its moisture.

Heat is transferred mostly by conduction from the hot surface of the pan through a thin layer of hot oil. So, the surface temperature of vegetable rises rapidly and a proportion of water is vaporised (Fellows, 2009). Consequently, this rapid heating will lead to myrosinase inactivation. Most of myrosinase from both vegetables is expected to be already inactivated at the beginning of stir-frying, resulting in a low influence on the GS hydrolytic degradation. Therefore, there was also no clear effect of various stir-frying times and temperatures on the GS changes. Stir-frying even could increase the extractability of the GS content, particularly for Chinese cabbage. The difference in plant matrix of Chinese cabbage and pakchoi may influence to the GS content after stir-frying. Dekker et al. (2009) and Sarvan et al. (2014) showed that thermal susceptibility of GSs in different *Brassica* vegetables is different, but remain similar in order of stability.

However, when considering the differences in the processing equipment, the processing method, and the ingredients involved in the process, the stir-frying method in this study does not completely respond to reality. So, the results should be further elaborated with the context of real practice of stir-frying. In Indonesia, stir-frying of vegetables also involves other ingredients, such as spices, garlic, and salt. Meat, seafood, other types of vegetable, and water may also be added during stir-frying. The molecular interaction between these ingredients may also influence the GS-myrosinase system of *Brassica* vegetable. Moreover, stir-frying is commonly performed by using a pan or wok on a stove instead of the electric one. Particularly in food service establishments, a high pressure burner is used and the flame level is adjusted manually. For the context of stir-frying time and temperature effect, it is likely that stir-frying employed in real situation can lead to the similar impact of the present study. Yet, all these complex factors bring a scientific challenge to performing further studies to integrate the technological perspective, sensorial preference, and behavioural aspects. These include studies on the effects of interaction between ingredient(s) and *Brassica* vegetables during stir-frying at various time and temperatures on GS content and evaluation of the consumers acceptance on the products, in terms of colour, texture, and taste. The identified critical quality attributes and processing conditions can be integrated by using e.g., consumer-oriented food technology approach (Bongoni et al., 2013) for multi-criteria optimization of vegetable quality, including health properties. Moreover, the result can be further integrated with other studies on behaviour of the food handlers, e.g., by using a techno-managerial approach (Luning and Marcelis, 2006), to analyse the feasibility of producing a good quality and healthy product.

Food service establishments, including street food vendors, have an important role on providing daily menu for society (Dawson and Canet, 1991). For households, the role of family member in food preparation is also vital. Therefore, practices of *Brassica* vegetable preparation in society should be taken into account. However, due to a high number of variations in each preparation method, the information on technical details of the method was not investigated. Nevertheless, the information on the preparation methods commonly performed by food handlers can be a useful approach to identify the implication of technology on the GS content (Chapter 3). This study found that both groups have different health perception on the preparation methods of vegetables. Moreover, certain preparation methods and *Brassica* vegetable types that are most frequently applied are not always perceived as the healthiest *Brassica* nor the preparation method to have highest health beneficial effect by the respondents. This might be due to the fact that in the (regular) practices, food handlers prepare and provide foods for consumers instead for themselves; except, probably for the family member in households. Hence, what kind of *Brassica* will be cooked and how to cook the *Brassica* have no relation with the health perception of the food handlers. Besides that, perception is influenced by many variables. Perception is a complex process of the senses and the brain. It is based on sensorial observations of individual and product characteristics, in this case the characteristics of *Brassica* vegetables. Moreover, experiences, atmosphere while perceiving the product, and indirect product characteristics like production methods can also influence the perception (Sijtsema et al., 2002). Therefore, further study is necessary to compare these two groups as well as the variation within the group with regards to the behaviour and motives of preparation of vegetable.

The behaviour and health perception related to food preparation may differ from one population group to another and differ over time. Hence, local conditions must be considered when applying the findings into society (Wahlqvist and Lee, 2007). Nevertheless, the description of preparation methods by food handlers may reflect a more general behaviour regarding vegetable preparation, particularly in Southeast Asia. Despite that preparation methods could reflect the diversity of food culture, globalisation of food (including the preparation of food) have been penetrating around the world. For instance, Paschel (2007) observed that people in the US evolve into a culture marked by global food preferences and consumers explore more global food preparations and flavour.

Implications and prospective studies

A mechanistic approach as applied in this research is demonstrated to be beneficial to identify the behaviour of GS in *Brassica* vegetables during preparation involving variable complexity. Variability of processing conditions that lead to these complexities can be elaborated by this approach to study GSs changes. For the *cabbage roll* and *sayur asin*, for instance, this finding can contribute to the design of production methods resulting in less GS degradation in these products. Moreover, by employing mathematical modelling based on the underlying mechanisms, the GS content in domestically prepared *Brassica* vegetables can be optimised. In practice however, the feasibility of these re-designing methods should be discussed thoroughly with the stakeholders, particularly the producers. As most of them are the small-scale food establishments, the modification should not affect significantly the cost, such as by not changing the processing equipment or providing measuring devices. The importance of simple measurements must be introduced, e.g., by indicating time/duration of each preparation step. Also, the implication for the profit or loss for the producer must be taken into account. From the consumer perspective, the sensorial properties, particularly colour, texture, and taste, should also be taken into account. Also, the degree of consumer's acceptance on the products containing health promoting compounds and the price should be considered. Thus, further studies on the producers' behaviour and motives as well as consumers preferences must be performed. Moreover, this study can be the basis for developing further studies on alleged health promoting compounds in Indonesian fruit and vegetables and the impact of processing.

It is demonstrated that processing can have a large impact on the behaviour and content of GSs. All variables contribute to the final intake of GSs and thus determine the health promoting potential of the food. However, GSs do not have health protection effect but the breakdown products. Therefore, it will be useful to further investigate the breakdown products of GSs in *Brassica* vegetables prior to consumption and the bioavailability of these compounds. However, it should take into account that the high number of heterogenic degradation and reaction products that might contribute to the bioactivity complicates the risk-benefit evaluation (Hanschen et al., 2014).

To our knowledge, this is the first study on the effects of preparation on GSs of *Brassica* vegetables in Indonesia, while previous studies were mainly performed in the Western perspective. Since the contribution of these vegetables is very important in daily diet of society, research along the production chain, from farm to table, will be highly valuable to contribute to formulate a strategy for promoting health from the side of food intake. For instance, the agriculture scientist can develop the *Brassica* vegetables containing significant amount of GSs by considering e.g., the conditions of soil, climate, and cultivars in the tropic. The influence of supply chain from farm to consumers prior to preparation should also be investigated as this can have substantial effect to the health promoting compounds.

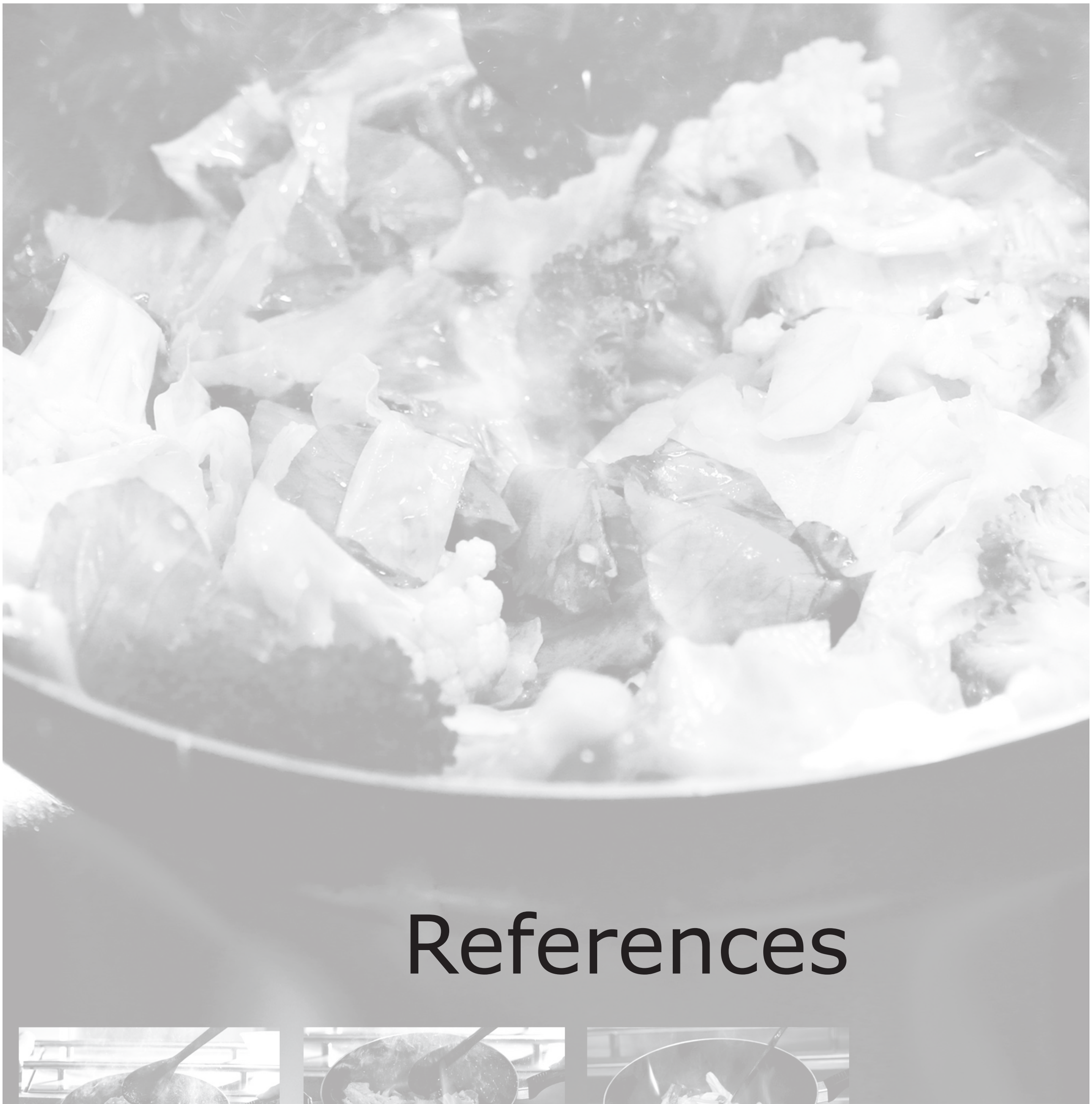
Although domestic preparation methods are still dominant for processing vegetables to fulfil the nutritional needs in Indonesia, the role of industrial processing becomes more pronounced. In domestic processing, it is more difficult to produce standardized product due to its high variability of materials and processing methods. For instance, the feasibility of *sayur asin* and other local fermented vegetables can be further investigated to improve the production from a domestic scale to an industrial processing scale.

The acquired knowledge and insights of this study open up possibilities to reformulate existing production methods and preparation ways for small-scale food establishments and households based on the various explained mechanisms responsible for the main GS losses. In term of producing the highest possible GS content in the product, this reformulation will be possible. However, with more phytochemicals in the product, then more comprehensive studies are needed prior to reformulation. Yet, since GSs are almost exclusively found in *Brassica* and considering the water-soluble and heat-degradable properties of GS, this study can be used as a valid foundation to reformulate a new product. In that way, a scientific approach to reformulate products and bring them to the market/ consumers can contribute to the aim of improving the Indonesian diet, while taking into account the limitations for this reformulation, such as the assumption(s) on whether the underlying mechanisms affect the GS losses and disregarding the variability of the food handlers' behaviour and consumers' preferences. A scientific approach still can give a substantial contribution provided that these limitations, especially consumers' preferences, are overcome. If not, then the reformulated product may fail in the market.

Main conclusions

The research reported in this thesis has yielded valuable knowledge on the GS content of *Brassica* products as consumed in Indonesia. By studying the different steps in the production chain it has become clear that some widely used preparation methods (long-term steaming and fermentation) result in a low retention of GS, while another widely used method in Indonesia, stir-frying, retains GS to a large extend. By analysing the preparation methods in terms of the mechanisms underlying the GS changes during processing and preparation it was shown to be possible to propose alternative procedures or conditions to enhance the retention of GS. This approach was demonstrated in this thesis for fermentation of *Brassica* vegetable, where prior inactivation of myrosinase resulted in a higher retention of GS. For steaming, a reduction of the steaming time was shown beneficial to have a higher GS content, while at the same time improving colour and texture of the product.

This thesis applied a mathematical modelling approach, based upon an understanding of the most relevant mechanisms in the process. This approach appeared to be a useful tool to describe the effects of variation in the conditions and processes on the GS content of products and optimise them in order to get a more optimal GS content in the prepared products. Besides improvement of the content of vegetable products, the present study also yields important information to make a more accurate estimation of the dietary intake of GSs in prepared dishes. Such estimation is important for establishing the relation between intake of phytochemicals and health effects like reducing the risk of certain diseases.



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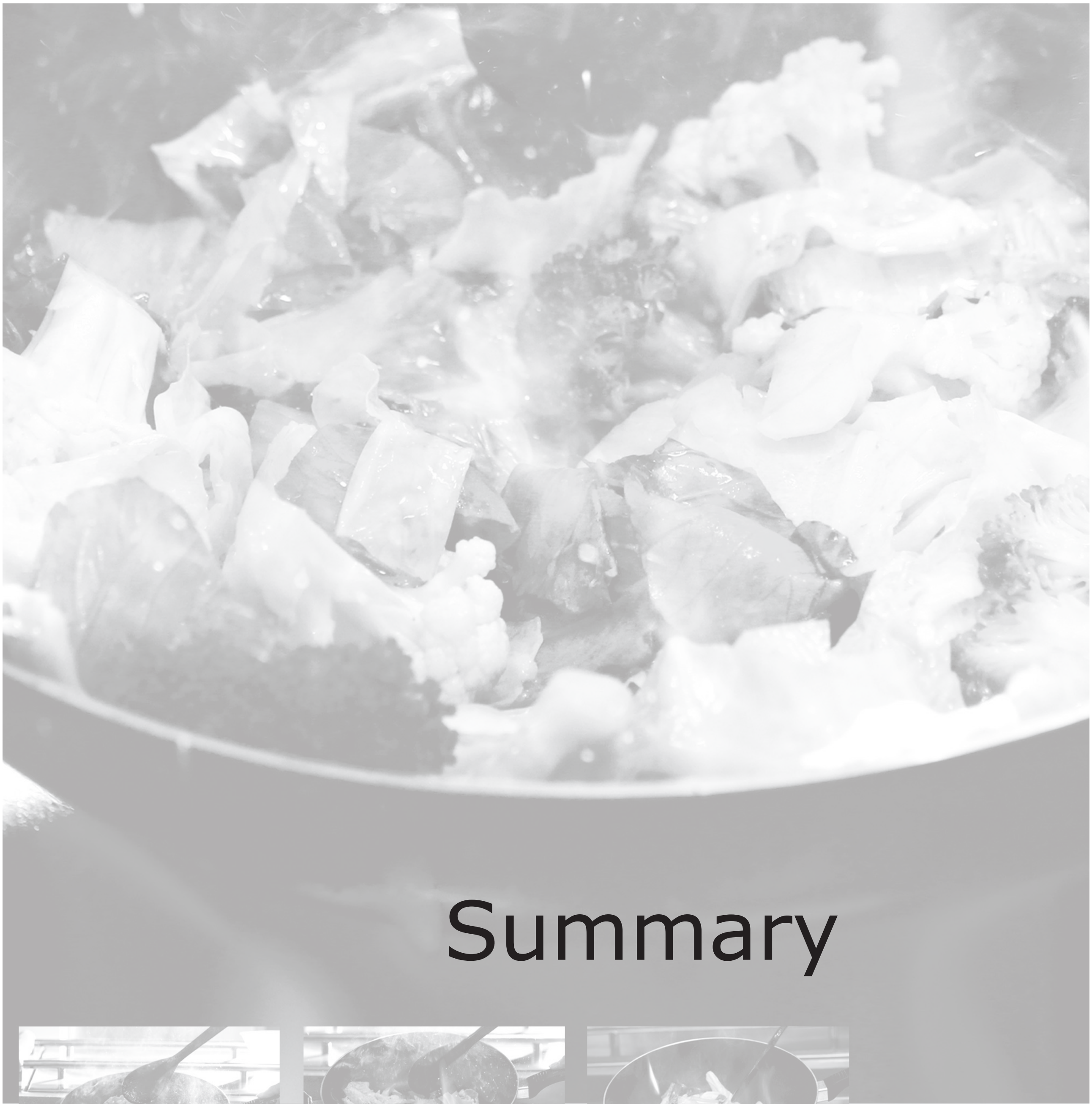


Summary

Background

Processing of *Brassica* vegetables can reduce the content of alleged health promoting glucosinolates (GSs). In order to make accurate estimates of dietary intake of these GSs, the effects of processing of *Brassica* vegetables should be taken into account. Various processing and domestic preparation methods can have different impacts on the GS content in the products. However, previous studies indicated complexities to compare and interpret the GS content as affected by various processing due to variability in processing conditions and analytical methods. Besides that, the studies are mainly performed in the context of Western preparation methods. The Southeast Asia countries, including Indonesia, have been producing large amounts of *Brassica* vegetables and the vegetables are prepared into various dishes and are consumed on a daily basis. Yet, there is no study on the effects of preparation methods commonly employed in Indonesia on GS content of *Brassica* vegetables.

Aims

The general objective of this thesis is to investigate the GS content of *Brassica* vegetables upon consumption by considering the various steps in the production chain and to investigate whether it is possible to optimize the GS content. More specifically, the effects of (Indonesian) preparation methods on the GS content of *Brassica* vegetables were studied by applying a mechanistic perspective that underlies the GS changes during preparation. These preparation methods are long-term steaming, fermentation, and stir-frying. Moreover, previous studies on the behaviour of GS content in *Brassica* vegetables as affected by various processing methods were also reviewed. To obtain information and knowledge on how the vegetables are commonly prepared, the preparation methods on *Brassica* vegetables and health perception among food handlers in food service establishments and households in Indonesia were also studied by a survey.

Results

Processing is one of the major factors affecting changes of GS content, mainly due to leaching and thermal degradation, along the production chain of *Brassica* vegetables. Publications on the effect of various processing and preparation methods on the GS content in *Brassica* vegetables were reviewed in **Chapter 2**. The changes of GSs were also analysed by discussing the relevant mechanisms underlying each processing method. Boiling and blanching generally reduce the GS content significantly due to cell lysis, diffusion, thermal degradation, and leaching. Meanwhile, steaming and microwave processing relatively retain or even increase the total accessible GS content because of a low impact of thermal degradation and leaching, and an apparent increase of the GS extractability in the plant matrix during analysis. Stir-frying slightly reduces the GS content. Changes of individual GSs are more complicated to be explained, involving individual GS properties in each type of *Brassica* vegetable and different responses to each preparation method and condition. This review showed the benefit of a mechanistic approach to discuss the GS changes as affected by domestic preparation methods.

Chapter 3 describes the preparation practices of food handlers in food service establishments and households in Semarang city, Indonesia and their perception of health benefits of these vegetables and preparation methods. The information on the preparation methods commonly performed by food handlers can be a useful approach to define the

implication of technology and alleged health promoting GSs. For preparation methods, boiling and stir frying are the most frequently applied for preparing *Brassica* vegetables. Meanwhile for *Brassica* types, white cabbage and choysum are the most frequently prepared vegetables compared to other *Brassica* vegetables. In terms of perception of health, broccoli is perceived by the respondents as the healthiest *Brassica*. Steaming and boiling are perceived to have the highest health beneficial effects according to the food service establishment respondents. Meanwhile, for the household respondents boiling and stir frying are perceived as the most health beneficial preparation methods. Thus, certain preparation methods and *Brassica* vegetable types that are most frequently applied are not always perceived as the healthiest *Brassica* nor the preparation method to have highest health beneficial effects by the respondents.

Studies on GS content as affected by three preparation methods commonly employed to produce local food products, namely steamed cabbage roll, fermented Indian mustard (*sayur asin*), and stir fried vegetable, are presented in Chapter 4 to Chapter 6. In **Chapter 4** the cabbage roll is prepared by a short blanching the leaf layers of cabbage followed by rolling and (long-term) steaming. The total accessible GSs after 10 min of steaming increased due to a higher extractability of GSs in the tissues. Subsequently, a consistent decline is observed until 180 min of steaming. This behaviour is contributed mainly to the aliphatic GSs, particularly glucoraphanin and glucobrassicin, rather than to the indolyls. The hydrolytic myrosinase had been inactivated by the blanching step prior to steaming and leaching into the condensed steam is expected at low level followed by drip loss. So, the decrease in GS content will be mainly caused by thermal degradation. Moreover, steaming for up to 10 min could also retain colour and texture of cabbage roll as important quality attributes. The mechanisms underlying long-term steaming were identified to explain the behaviour of GS content (i.e. glucoraphanin) by using mathematical modelling approach. The mathematical model fits the observed concentration profile during the steaming.

Myrosinase activity is one of the factors strongly underlying the changes of GS during processing of *Brassica* vegetable. The effect of myrosinase activity was studied during fermentation of Indian mustard (*Brassica juncea*) in the production of *sayur asin* (**Chapter 5**). Sinigrin is the most dominant GS in the raw Indian mustard, accounting for about 95% of the total GSs. Myrosinase activity had significance influence on GS content of Indian mustard during fermentation. Withering the leaves by oven at 35 ˚C and by microwave for 4.5 minutes at 180 W retained the myrosinase activity at 16 % and 26 %, respectively. After a microwave treatment for 2 minutes at 900 W no myrosinase activity was detected in the leaves. Withering and fermentation substantially reduced the GS content when active myrosinase is present. During fermentation, the reduction is affected by the levels of myrosinase activity from the withered leaves. GSs in *sayur asin* are not detectable after the third day of fermentation, when active myrosinase is still present in the withered leaves prior to fermentation. The *sayur asin* of fermentation day 7, which contained inactivated myrosinase, retained the GS sinigrin at about 11.4 µmol/ g DM. It was also found that lactic acid bacteria are mainly responsible for the fermentation of *sayur asin*.

Chapter 6 presents the effect of stir frying on the GSs of Chinese cabbage (*Brassica rapa* ssp *pekinensis*) and pakchoi (*Brassica rapa* ssp *chinensis*), by varying cooking parameters, namely time and temperature. Aliphatic glucobrassicanapin is the most abundant GS identified in both *Brassica rapa* species, followed by glucoiberin and gluconapin. Stir frying was shown to retain the GS content and even could increase the extractability of the GS content, particularly for Chinese cabbage. There was no clear effect of time and temperature of stir frying on the GS changes. Due to the absence of a separate water phase, leaching losses did not occur. The retention the GS content in Chinese cabbage and pakchoi indicate the low degrees of cell lysis and diffusion, leaching, thermal degradation, and enzymatic hydrolysis mechanisms. The observed high retention of GSs during stir frying are comparable to short time steaming and microwave processing of *Brassica* vegetables.

Conclusions

Mechanistic approach is demonstrated to be beneficial to identify the behaviour of GS in *Brassica* vegetables during preparation commonly performed in Indonesia. Long-term steaming and fermentation result in a low retention of GS, while stir-frying retains GS to a large extend. By analysing the preparation methods in terms of the mechanisms underlying the GS changes during processing and preparation it was shown to be possible to propose alternative procedures or conditions to enhance the retention of GS. This approach was demonstrated in this thesis for fermentation of *Brassica* vegetable, where prior inactivation of myrosinase resulted in a higher retention of GS. For steaming, a reduction of the steaming time was shown beneficial to have a higher GS content, while at the same time improving colour and texture of the product.

This thesis applies a mathematical modelling approach, based upon an understanding of the most relevant mechanisms in the process. This approach is a useful tool to describe the effects of variation in the conditions and processes on the GS content of products and optimise them in order to get a more optimal GS content in the prepared products. Besides improvement of the content of vegetable products, the present study also yields important information to make a more accurate estimation of the dietary intake of GSs in prepared dishes. Such estimation is important for establishing the relation between intake of phytochemicals and health effects like reducing the risk of certain diseases.

Samenvatting



Achtergrond
Verwerking en bereiding van Brassica groenten kan de hoeveelheid aan gezondheidsbevorderende glucosinolaten (GSs) verlagen. Om nauwkeurige schattingen te maken van de inname aan GSs is het van belang om het effect van de bereiding van koolsoorten mee te nemen. Verschillende verwerkingsprocessen en huishoudelijke bereidingsmethoden kunnen verschillende effecten hebben op de GS gehalten in de groenten. Eerdere studies hebben aangegeven dat het GS gehalte sterk varieert door de diverse bereidingswijzen en de verschillende analytische methoden. Tevens zijn deze studies voornamelijk uitgevoerd met Westerse bereidingsmethoden van de Brassica groenten. Aziatische landen, waaronder Indonesië, produceren grote hoeveelheden aan diverse Brassica groenten die in verschillende gerechten worden gebruikt en dagelijks worden geconsumeerd. Toch zijn er weinig studies uitgevoerd naar de effecten van Aziatische bereidingswerkwijzen op de gehaltes glucosinolaten in koolsoorten.

Doelstellingen
De hoofddoelstelling van dit proefschrift is om het effect van verschillende stappen in de productieketen van Brassica groenten op het GS gehalte te onderzoeken. In het bijzonder worden de effecten van Aziatische (Indonesische) bereidingsmethoden bestudeerd door mechanismen te onderzoeken die de veranderingen in GS gehaltes kunnen verklaren tijdens de bereiding van groenten. De onderzochte bereidingswijzen zijn: langdurig stomen, fermentatie, en roerbakken. Een literatuurstudie is uitgevoerd om een overzicht te geven van verschillende bereidingswijzen en de effecten hiervan op glucosinolaten. Door middel van enquêtes onder eetgelegenheden en huishoudens in Indonesië is informatie en kennis verkregen over de gangbare bereidingswijzen van Brassica groenten en de perceptie op gezondheid van de gerechten.

Resultaten
Verwerking is één van de belangrijkste factoren die verantwoordelijk is voor afnames van de GS gehaltes in de productieketen van Brassica groenten. Dit wordt voornamelijk veroorzaakt door het uitlekken en thermische afbraak van deze componenten. Een literatuurstudie naar de effecten van verschillende verwerkings- en bereidingsmethoden op het GS gehalte in Brassica groenten is beschreven in **Hoofdstuk 2**. Verschillende mechanismen die mogelijk verantwoordelijk zijn voor de veranderingen in GS gehaltes worden hierbij besproken. Koken en blancheren verlagen het GS gehalte aanzienlijk als gevolg van cellysis, diffusie, thermische afbraak, en uitlekken van de componenten. Stomen en magnetronbereiding van groenten behouden de GS gehaltes of kunnen zelfs een hoger beschikbaar GS gehalte geven door een geringe thermische afbraak en uitlek, en een duidelijke toename van de beschikbare GS hoeveelheid uit de plant matrix tijdens de analyse. Roerbakken zorgt voor een kleine verlaging van het GS gehalte. De literatuurstudie geeft het belang aan om inzicht te verkrijgen in diverse mechanismen die een rol spelen bij de verandering in GS gehaltes tijdens de verschillende bereidingswijzen van Brassica groenten.

Hoofdstuk 3 beschrijft de bereidingswijzen van groenten door personeel in eetgelegenheden en huishoudens in Semarang stad, Indonesië, waarbij tevens hun perceptie van de gezondheid van deze groenten en de bereidingswijzen is onderzocht. De meest toegepaste bereidingswijzen van Brassica groenten zijn koken en roerbakken, waarbij witte kool en choysum de meest gebruikte groenten zijn. Broccoli wordt gezien als de meest gezonde groente, terwijl koken en stomen als de meest gezonde bereidingswijzen worden gezien door de voedselbereiders in eetgelegenheden. Voor huishoudens zijn koken en roerbakken de meest gezonde manieren van groente bereiding. Kortom, de meest gebruikte soorten Brassica groenten en bereidingswijzen worden niet altijd gezien als de meest gezonde.

De studies uitgevoerd naar drie lokale bereidingswijzen namelijk stomen van "kool-rolls", fermentatie van *sayur asin* en roerbakken van groenten, worden gepresenteerd in respectievelijk **Hoofdstuk 4, 5 en 6**. In **Hoofdstuk 4** is de bereiding van "kool-rolls" beschreven na een korte blancheer stap van de witte koolbladeren, gevolgd door een langdurige stoombehandeling. De totale beschikbare hoeveelheid glucosinolaten in de "kool-rolls" nam toe na 10 minuten stomen, wat waarschijnlijk toe te schrijven is aan een toename in de extraheerbaarheid van de glucosinolaten uit de matrix. Vervolgens werd een geleidelijke afname in het GS gehalte waargenomen gedurende de resterende 180 minuten stoomtijd. De verandering in totaal GS gehalte wordt voornamelijk toegeschreven aan de alifatische glucosinolaten en in veel mindere mate aan de indolyl GS. Het hydrolytische enzym myrosinase is geïnactiveerd door de blancheerstap voorafgaand aan het stomen van de "rolls" en het mechanisme van uitlekken in de gecondenseerde stoom gevolgd door terugvloeien van deze vloeistof wordt verwacht een relatief laag verlies te geven. De afname in GS gehalte zal daarom voornamelijk plaatsvinden door thermische afbraak van de glucosinolaten tijdens blancheren en stomen. De belangrijke kwaliteitsattributen kleur en textuur van de koolbladeren werden behouden gedurende de eerste 10 minuten van het stomen. De belangrijkste mechanismen van de veranderingen van het GS (glucoraphanin) gehalte zijn gebruikt om dit wiskundig te modelleren tijdens het langdurige stomen van de "kool-rolls". Dit wiskundig model beschrijft het waargenomen concentratie profiel van glucoraphanin tijdens het stomen.

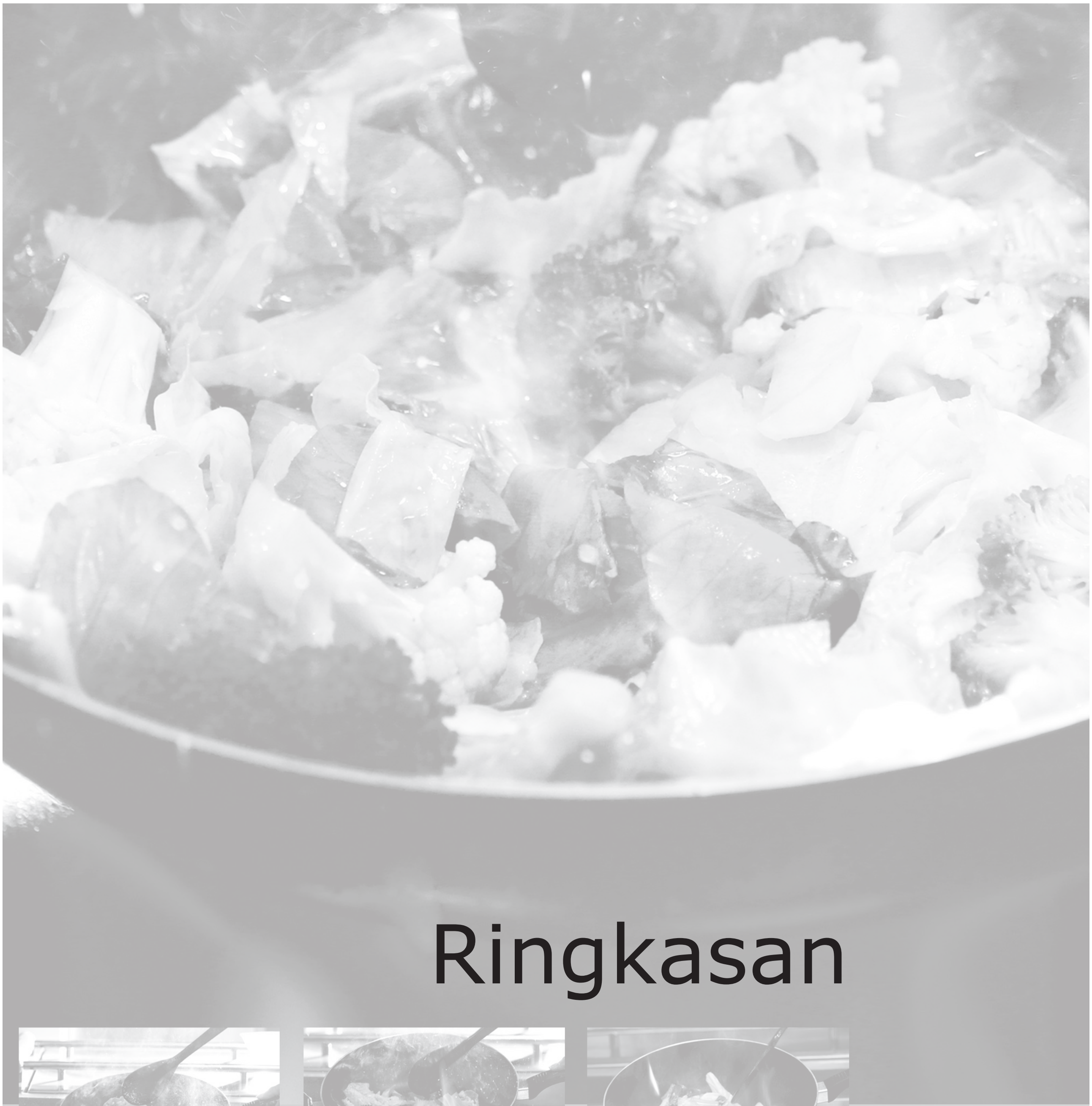
Myrosinase activiteit is een belangrijke factor bij de veranderingen van het GS gehalte tijdens de verwerking van groenten. De rol van myrosinase is onderzocht tijdens de fermentatie van Indian mustard (*Brassica juncea*) tijdens de bereiding van *sayur asin* (**Hoofdstuk 5**). Sinigrine is het meest dominante GS in de rauwe Indian mustard bladeren, verantwoordelijk voor 95% van het totaal GS gehalte. Aangetoond is dat myrosinase activiteit een substantiële invloed heeft op het GS gehalte tijdens het fermentatie proces. De voorbehandeling, het laten verwelken van de *B. juncea* bladeren in een oven bij 35 ˚C of in een magnetron gedurende 4,5 minuut bij 180 W, behield de myrosinase activiteit met respectievelijk 16% en 26 %. Een magnetron behandeling van 2 minuten bij 900W resulteerde in een volledig inactief myrosinase in de bladeren. Het verwelken en de fermentatie van de Brassica bladeren resulteerde, in aanwezigheid van een actief myrosinase, in een aanzienlijk verlaging van het GS gehalte. Tijdens de fermentatie is de afname in GSs afhankelijk van de mate van myrosinase activiteit in de verwelkte bladeren. In aanwezigheid van een actief enzym zijn er geen GSs te detecteren na 3 dagen fermentatie. Na 7 dagen fermentatie bevat de *sayur asin,* waarin myrosinase geïnactiveerd was, ongeveer 11,4 µmol/ g DM sinigrine. Melkzuurbacteriën waren voornamelijk verantwoordelijk voor het fermentatieproces.

In **Hoofdstuk 6** zijn de effecten van roerbakken van Chinese kool (*Brassica rapa* ssp *pekinensis*) en pakchoi (*Brassica rapa* ssp *chinensis*) op de GS gehaltes beschreven voor verschillende tijden en temperaturen. Het alifatische glucosinolaat glucobrassicanapin is het meest voorkomende GS in beide *Brassica rapa* soorten, gevolgd door glucoiberin en gluconapin. Het is gebleken dat roerbakken de glucosinolaten in de groenten kan behouden en zelfs een toename kan laten zien in de beschikbare GSs, voornamelijk bij Chinese kool, waarschijnlijk veroorzaakt door een betere extractie uit de matrix. Er was geen eenduidig effect van tijd en temperatuur van het roerbakken op de GS gehaltes. Het uitlekken van GSs tijdens roerbakken zal beperkt zijn door de afwezigheid van toegevoegd water. De relatief hoge retentie van GSs in Chinese kool en pakchoi duidt er op dat er tijdens het roerbakken sprake is van een beperkte mate van uitlekken, thermische afbraak en enzymatische hydrolyse van GSs. De waargenomen retentie van GSs bij het roerbakken is vergelijkbaar met de resultaten bij kort stomen en magnetronbereiding van *Brassica* groenten.

Conclusies

Een mechanistische benadering is succesvol gebleken om het gedrag van GSs tijdens veelgebruikte Indonesische bereidingsmethoden te beschrijven. Langdurig stomen en fermenteren resulteren in lage retenties van GSs, terwijl roerbakken een hoge retentie laat zien. Door deze bereidingsmethoden te analyseren aan de hand van de onderliggende mechanismes van de GSs veranderingen tijdens verwerking en bereiding was het mogelijk om alternatieve procedures of condities voor te stellen om de retentie van GSs te verhogen. Deze benadering is in dit proefschrift gedemonstreerd voor de fermentatie van Brassica groente, waarbij een voortijdig inactiveren van myrosinase leidde tot een hogere retentie van GSs. Bij stomen van Brassica groenten was het verkorten van de stoomtijd gunstig voor een hoger GSs gehalte, terwijl ook de kleur en de textuur van het product verbeterden.

Dit proefschrift heeft een wiskundige modelleer benadering toegepast, gebaseerd op het begrip van de meest relevante mechanismes tijdens de processen. Dit is een nuttige benadering om de effecten van de variaties in de procescondities op de GS gehalten te beschrijven en te optimaliseren om te komen tot een optimaal GS gehalte in de bereidde producten. Naast het verbeteren van de gehaltes in de groenten heeft dit onderzoek ook belangrijke informatie opgeleverd om een nauwkeurigere schatting van de inname van GSs in bereidde producten te kunnen maken. Een dergelijke schatting is belangrijk om de relatie tussen de inname van phytochemicaliën en de gezondheidseffecten (zoals het reduceren van de kans op bepaalde ziekten) vast te kunnen stellen.



Ringkasan



Latar belakang
Pengolahan sayuran jenis *Brassica* dapat menurunkan kandungan berbagai glukosinolat (GS), suatu kelompok senyawa yang dapat meningkatkan kesehatan, yang ada pada sayuran tersebut. Oleh karena itu, pengaruh pengolahan pada sayuran *Brassica* harus diperhatikan untuk dapat memberikan gambaran estimasi asupan diet GS yang akurat. Berbagai cara pengolahan dapat memberi dampak yang beragam terhadap kandungan GS di dalam produk. Namun, hasil-hasil penelitian tentang pengaruh cara-cara pengolahan terhadap kandungan GS terlalu kompleks untuk dapat dibandingkan dan diinterpretasi karena keragaman kondisi pengolahan dan metode analisis. Selain itu, penelitian tersebut kebanyakan dilakukan dalam konteks cara pengolahan di negara-negara barat. Kawasan Asia Tenggara, termasuk Indonesia, juga merupakan penghasil sayuran *Brassica* dalam jumlah yang besar dan sayuran ini diolah menjadi aneka jenis makanan atau hidangan dan biasa dikonsumsi sehari-hari. Sayangnya, belum ada penelitian tentang pengaruh berbagai metode pengolahan sayuran *Brassica* yang biasanya dilakukan di Indonesia terhadap kandungan GS pada produk.

Tujuan
Secara umum, tesis ini bertujuan untuk meneliti kandungan GS pada sayuran *Brassica* saat dikonsumsi dengan mempertimbangkan berbagai tahapan dalam rantai produksi dan mengkaji peluang untuk melakukan optimalisasi kandungan GS dalam produk. Secara lebih khusus, dilakukan penelitian tentang pengaruh berbagai metode pengolahan sayuran *Brassica* di Indonesia terhadap kandungan GS dengan menerapkan perspektif mekanistik yang mendasari perubahan-perubahan kandungan GS selama pengolahan. Metode pengolahan ini meliputi pengukusan, fermentasi, dan penumisan/ pemasakan ca. Selain itu juga dilakukan tinjauan pustaka terhadap berbagai publikasi hasil penelitian tentang pengaruh berbagai metode pengolahan terhadap kandungan GS dalam sayuran *Brassica*. Untuk memperoleh informasi tentang praktik pengolahan sayuran itu, dilakukan juga survei tentang berbagai metode pengolahan sayuran *Brassica* dan persepsi kesehatan di kalangan penyedia jasa/ pengolah makanan dan rumah tangga di kota Semarang, Indonesia.

Hasil
Pengolahan adalah salah satu faktor utama yang mempengaruhi perubahan kandungan GS di sepanjang rantai produksi sayuran *Brassica*, terutama karena senyawa tersebut terikut ke dalam air dan rusak karena panas. Berbagai publikasi tentang pengaruh berbagai metode pengolahan terhadap kandungan GS di sayuran *Brassica* ditinjau di **Bab 2**. Berbagai perubahan pada GS juga dibahas melalui berbagai mekanisme yang relevan, yang mendasari tiap-tiap metode pengolahan. Perebusan dan blansing/blansir umumnya menurunkan kandungan GS di dalam sayuran karena adanya kerusakan sel pada jaringan, difusi, kerusakan GS akibat suhu tinggi, dan terikutnya GS ke dalam air. Sementara itu, pengukusan dan pengolahan menggunakan gelombang-mikro/*microwave* relatif mempertahankan atau bahkan meningkatkan kandungan GS yang dapat terukur karena rendahnya dampak kerusakan akibat panas dan kecilnya peluang GS terikut ke dalam air, serta meningkatnya kemampuan GS terekstrak dari dalam matriks sayuran selama analisis. Sedangkan penumisan dapat sedikit mempengaruhi penurunan kandungan GS. Perubahan pada setiap individu GS lebih kompleks untuk dapat dibahas, karena melibatkan sifat dari masing-masing GS di setiap jenis sayuran *Brassica* dan hasil yang sangat beragam untuk setiap metode dan kondisi pengolahan. Hasil tinjauan

ini menunjukkan bahwa pendekatan mekanistik bermanfaat dalam membahas berbagai perubahan GS yang disebabkan oleh berbagai metode pengolahan domestik.

Bab 3 menggambarkan praktik pengolahan sayuran *Brassica* oleh para pengolah makanan di penyedia jasa makanan dan rumah tangga di kota Semarang, Indonesia dan persepsi mereka tentang manfaat kesehatan sayuran tersebut dan metode pengolahannya. Pendekatan melalui survei tentang metode pengolahan yang biasanya dilakukan oleh para pengolah makanan berguna untuk mengkaji dampak teknologi terhadap kandungan senyawa GS. Dari survei tersebut didapatkan bahwa perebusan dan penumisan merupakan metode yang paling sering dilakukan untuk mengolah sayuran *Brassica*. Sementara itu, kol/kubis dan caisim merupakan sayuran yang paling sering diolah dibandingkan sayuran *Brassica* lainnya. Terkait persepsi kesehatan, brokoli dipersepsi oleh para responden sebagai sayur *Brassica* yang paling menyehatkan. Selanjutnya, pengukusan dan perebusan dipersepsi sebagai metode pengolahan yang memberikan manfaat kesehatan tertinggi menurut responden dari kalangan penyedia jasa makanan. Sedangkan bagi para responden rumah tangga, perebusan dan penumisan dipersepsi sebagai metode pengolahan yang memberikan manfaat kesehatan tertinggi. Jadi, baik metode pengolahan tertentu yang paling sering dilakukan maupun jenis sayur *Brassica* tertentu yang paling sering diolah tidak selalu dipersepsi sebagai metode pengolahan dan sayur *Brassica* yang paling besar manfaatnya dari segi kesehatan.

Penelitian tentang kandungan GS pada tiga olahan sayur *Brassica*, yaitu kubis gulung kukus, sayur asin, dan tumis sayur, ditunjukkan di Bab 4 hingga Bab 6. Di **Bab 4**, kubis gulung kukus dibuat dengan cara blansing lembaran-lembaran kubis diikuti dengan penggulungan, dan pengukusan dalam jangka waktu yang lama. Setelah pengukusan selama 10 menit, kandungan GS total (yang dapat terukur) meningkat karena kemampuan GS di dalam jaringan untuk dapat terekstrak juga meningkat. Selanjutnya, penurunan GS terjadi hingga pengukusan selama 180 menit. Pola ini terutama disokong oleh GS golongan alifatik, khususnya glukorafanin dan glukobrasicin, daripada GS golongan indole. Enzim myrosinase terinaktivasi pada saat blansing, sebelum pengukusan. Peluang terikutnya GS ke dalam uap panas yang terkondensasi dan GS hilang juga kecil. Jadi, penurunan kandungan GS lebih disebabkan oleh kerusakan akibat suhu tinggi. Sementara itu, pengukusan selama 10 menit juga dapat mempertahankan warna dan tekstur (kekerasan) kubis gulung kukus. Berbagai mekanisme yang terjadi selama pengukusan (dalam jangka waktu yang lama) kemudian diidentifikasi untuk menjelaskan perilaku kandungan GS (yaitu glukorafanin) dengan menggunakan pemodelan matematika. Model matematika yang digunakan sudah cocok dengan profil konsentrasi GS yang diukur selama pengukusan.

Aktivitas myrosinase merupakan salah satu faktor utama yang menyebabkan perubahan GS selama pengolahan sayuran *Brassica*. Pengaruh aktivitas myrosinase selama fermentasi sawi pahit (*Brassica juncea*) selama produksi sayur asin diteliti (**Bab 5**). Sinigrin adalah GS yang paling dominan di sawi pahit mentah, yang menyusun sekitar 95% dari GS total. Aktivitas myrosinase memberikan pengaruh yang nyata terhadap perubahan kandungan GS di sawi pahit selama fermentasi. Pelayuan sawi pahit menggunakan oven pada suhu 35 ˚C dan menggunakan gelombang-mikro selama 4,5 menit pada 180 W mampu mempertahankan aktivitas myrosinase berturut-turut sebesar 16% dan 26%. Sedangkan perlakuan selama 2 menit pada 900 W memberikan pengaruh terhadap tidak adanya aktivitas myrosinase pada sawi pahit layu. Pelayuan dan fermentasi menurunkan kandungan GS secara nyata ketika myrosinase masih aktif di dalam sawi pahit. Selama fermentasi, penurunan GS disebabkan oleh seberapa besar aktivitas myrosinase di sawi pahit layu. Setelah tiga hari fermentasi, kandungan GS dalam sayur asin tidak dapat terdeteksi pada sampel dengan myrosinase masih aktif dalam sawi pahit layu sebelum fermentasi. Sayur asin hasil dari fermentasi hari ke -7, yang mengandung myrosinase yang sudah tidak aktif, dapat mempertahankan kandungan sinigrin sebesar 11,4 µmol/g berat kering. Penelitian ini juga menunjukkan bahwa bakteri asam laktat memiliki peran utama dalam fermentasi sayur asin.

Bab 6 memaparkan hasil penelitian tentang pengaruh berbagai kombinasi suhu dan waktu penumisan terhadap kandungan GS dalam sawi putih (*Brassica rapa* ssp *pekinensis*) dan sawi sendok (*Brassica rapa* ssp *chinensis*). Glukobrasikanapin merupakan GS golongan alifatik yang paling banyak terdapat di kedua jenis *Brassica rapa* tersebut, diikuti dengan glukoiberin dan glukonapin. Penumisan dapat mempertahankan kandungan GS dan bahkan dapat meningkatkan kemampuan terekstraknya GS, khususnya pada sawi putih. Namun, waktu dan suhu penumisan tidak memberikan pengaruh yang jelas terhadap perubahan kandungan GS. Selain itu, peluang kehilangan GS karena terikut ke dalam air juga tidak terjadi karena tidak adanya penambahan air selama penumisan. Ketahanan kandungan GS dalam sawi putih dan sawi sendok mengindikasikan bahwa mekanisme kerusakan sel dan difusi, terikutnya GS dalam air, kerusakan akibat suhu tinggi, dan kerusakan enzimatis terjadi pada tingkat yang rendah. Ketahanan yang tinggi dari GS selama penumisan ini dapat dikatakan setara dengan hasil pengolahan pengukusan dalam jangka waktu pendek dan dengan gelombang-mikro.

Kesimpulan

Pendekatan mekanistik terbukti memberikan manfaat untuk mengidentifikasi perilaku GS di sayuran *Brassica* selama pengolahan yang biasa dilakukan di Indonesia. Pengukusan dalam jangka waktu yang lama dan fermentasi berakibat pada turunnya kandungan GS, sedangkan penumisan dapat mempertahankan kandungan GS. Melalui analisis terhadap berbagai metode pengolahan berdasarkan pada berbagai mekanisme yang mempengaruhi perubahan GS selama pengolahan, dapat diusulkan prosedur atau kondisi alternatif untuk meningkatkan ketahanan GS. Tesis ini juga menunjukkan bahwa pada fermentasi sayuran *Brassica*, ketahanan GS yang lebih tinggi dapat dicapai melalui perlakuan inaktivasi myrosinase sebelum fermentasi. Sedangkan pada pengukusan, penurunan waktu pengukusan memberikan keuntungan berupa kandungan GS yang lebih tinggi, sekaligus juga menjaga mutu warna dan tekstur pada produk.

Tesis ini juga menerapkan pendekatan pemodelan matematika, berdasarkan pada pemahaman tentang berbagai mekanisme yang paling relevan di dalam setiap proses pengolahan. Pendekatan ini berguna untuk menjelaskan pengaruh keragaman kondisi dan cara pengolahan terhadap kandungan GS pada produk dan untuk mengoptimalkannya, sehingga pada akhirnya dapat dihasilkan produk dengan kandungan GS yang lebih optimum. Selain itu, penelitian ini juga memberikan informasi yang berguna untuk melakukan estimasi asupan diet GS yang lebih akurat. Estimasi ini penting untuk mengetahui hubungan antara asupan berbagai senyawa fitokimia dan pengaruhnya terhadap kesehatan, seperti misalnya untuk menurunkan risiko penyakit tertentu.

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About the Author

Curriculum vitae

Robertus Probo Yulianto Nugrahedi was born on 25 July 1975 in Pati, Central Java, Indonesia. He finished his Bachelor of science in Food Technology at Gadjah Mada University, Yogyakarta, in 1999. After working at a food company, then, at one private university in Surakarta, in 2001 he started to work at the Department of Food Technology, Soegijapranata Catholic University (SCU) in Semarang as a junior/assistance lecturer. In 2002–2003, Probo was awarded with the Huygens scholarship from Nuffic to perform a research on the respiration of tomato during pre- and post-harvesting, at the Trace Gas research group, Radboud University, The Netherlands (formerly KUN). In 2004–2006 he got the StuNed scholarship to take his MSc study in Food Technology at Wageningen University, The Netherlands. In this period he joined with the Product Design and Quality Management group (now FQD), working for his thesis on the quality of mango puree during storage. In 2009 he started his PhD study, financially supported by the Directorate General for Higher Education, Indonesia. From 2001 to date, he has been working at the Department of Food Technology, Soegijapranata Catholic University, Indonesia and currently appointed as the vice dean for student affairs.

List of Publications (2009 – 2015)

<u>in peer-reviewed journals</u>

- Nugrahedi, P.Y., Verkerk, R., Widianarko, B., and Dekker, M. (2015). A mechanistic perspective on process-induced changes in glucosinolate content in *Brassica* vegetables: a review. *Critical Reviews in Food Science and Nutrition* 55: 823–838

- Nugrahedi, P.Y., Widianarko, B., Dekker, M., Verkerk, R., and Oliviero, T. (2015). Retention of glucosinolates during fermentation of *Brassica juncea*: a case study on production of *sayur asin*. *European Food Research and Technology* 240: 559–565

<u>Submitted</u>

- Nugrahedi, P.Y., Dekker, M., Widianarko, B., and Verkerk, R. Quality of cabbage during long-term steaming; phytochemical, texture, and colour evaluation.

- Nugrahedi, P.Y., Hantoro, I., Verkerk, R., Dekker, M., and Steenbekkers, L.P.A. Practices and health perception of preparation of *Brassica* vegetables: translating survey data to technological and nutritional implications.

- Nugrahedi, P.Y., Oliviero, T., Heising, J.K., Dekker, M., and Verkerk, R. Stir-frying retains glucosinolate content in Chinese cabbage (*Brassica rapa ssp pekinensis*) and pakchoi (*Brassica rapa ssp chinensis*)

<u>in Indonesia peer-reviewed journals</u>

- Nugrahedi, P.Y., Pranoto, P.Y., and Hantoro, I. (2010). *Dari Darum ke Kanjengan: penanganan, mutu, and persepsi mutu kubis di sepanjang rantai pasokan* (From Darum to Kanjengan: handling, quality, and quality perception of white cabbage along supply chain). *Jurnal Renai* 10(1): 50–63 (in *Bahasa Indonesia*)

- Nugrahedi, P.Y., Priatko, C.A., Verkerk, R., Dekker, M., and Widianarko, B. (2013). Reduction of glucosinolate content during *sayur asin* fermentation. *Jurnal Teknologi dan Industri Pangan* 24: 235–239

- Dukut, E.M., Utami, M.P., Nugroho, A., Putri, N.I. and <u>Nugrahedi, P.Y.</u> (2014). Using popular culture's media of Indonesian-English picturebooks as a way of reaching more vegetable consuming children. *CELT-Journal of Culture, English Language Teaching & Literature* 14: 36–47

<u>Conferences</u>

- Nugrahedi, P.Y., Putri, N.I., Verkerk, R., Dekker, M., and Widianarko, B. (2013) Integrating research in food and health: a case of promoting health by glucosinolates in *Brassica* vegetables. International conference on environment and health, Semarang, Indonesia, 22–23 May 2013.

- Nugrahedi, P.Y., Putri, N.I., Priatko, C.A., Verkerk, R., Dekker, M., and Widianarko, B. Glucosinolate behaviour in *Brassica* vegetables during steaming and fermentation. National seminar of food science and technology (IAFT), Jember, Indonesia, 28–31 August 2013 (poster)

- Nugrahedi, P.Y., Widianarko, B., Dekker, M., Verkerk, R., and Oliviero, T. (2014). Effect of myrosinase activity on glucosinolate content along the production of *sayur asin*. International conference on sustainable global agriculture & food security, Bangkok, Thailand, 16–18 July 2014

Picturebooks

Dukut, E.M., Putri, N.I., Utami, M.P., Nugrahedi, P.Y., and Nugroho, A. (2014) I love vegetable book series for children. Gramedia Pustaka Utama Publ., Jakarta (Bi-lingual in *Bahasa Indonesia* & English).

List of the book titles :

- Rocco the Broccoli Rocker
- Poki the Pakchoy Chef
- Spibam the Super Spinach
- Tommy the Tomato Actor
- Kartini the Carrot Dancer

Overview of completed training activities

Discipline specific activities : Courses

- Starting with the client: new approaches to effective health promotion, VLAG, 2009
- Advanced food analysis, VLAG, 2010
- Food safety management system & HACCP, Soegijapranata Catholic University (SCU) & SAI Global, 2011
- Food packaging and shelf life, SCU, 2014

Discipline specific activities : Meetings

- SCU seminar on Agriculture and poverty: toward a fair/just food supply chain, SCU & Percik, 2010 (oral)
- SCU seminar on Indonesia higher education, 2012 (poster)
- International conference on environment and health, United Board for Christian Higher Education in Asia & SCU, 2013 (oral)
- Annual student conference on Food Science and Technology, Food Technology Department (FT) SCU, 2009–2012 (oral & poster)
- Indonesia Association of Food Technologists conference, IAFT, 2013 (poster)
- International conference on sustainable global agriculture & food security, Bangkok, 2014 (oral)

General courses

- VLAG PhD week, 2010
- Techniques for writing and presenting a scientific paper, WGS, 2010
- Information literacy including EndNote introduction, Wageningen University (WU), 2010
- Future defence leader workshop, Ministry of Defence Indonesia, 2010
- Upgrading teaching techniques in English, SCU & De La Salle University, 2013

Optionals

- Preparation PhD research proposal, 2009
- Food Quality Management course, FQD, WU, 2010
- PhD group discussion, FQD, WU, 2009-2013
- PhD trip to Australia, FQD, 2010
- FT-SCU half-day seminars on food & society, 2011–2012
- IAFT chapter Semarang meetings, 2012–2013

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