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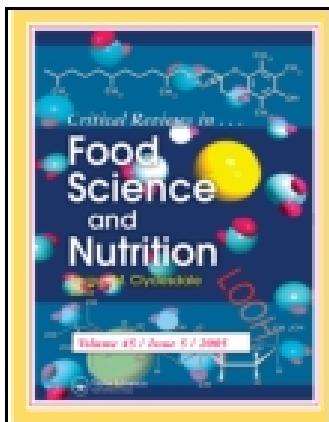
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### A Mechanistic Perspective on Process-Induced Changes in Glucosinolate Content in Brassica Vegetables: A Review

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# A Mechanistic Perspective on Process-Induced Changes in Glucosinolate Content in *Brassica* Vegetables: A Review

PROBO Y. NUGRAHEDI,<sup>1,2</sup> RUUD VERKERK,<sup>1</sup> BUDI WIDIANARKO,<sup>2</sup>  
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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 glucosinolates 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 glucosinolates 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** Glucosinolates, *Brassica* vegetable, processing, mechanistic approach, health

## INTRODUCTION

Vegetables of *Brassica* sp, 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 (GLSs), 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).

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*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 GLS content along the production chain (Dekker et al., 2000). Various reviews have briefly discussed the effects of processing on GLS 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 GLSs in *Brassica* during processing is

approximately 36%. More recently, Ruiz-Rodriguez et al. (2008) summarized the effects of some domestic processing methods on GLS content qualitatively and indicated steaming is the best method retaining the GLS content. Moreover, Rungapamestry et al. (2007) reviewed the effects of boiling, blanching, steaming, and microwave processing on GLS content, myrosinase activity, and the formation and bioavailability of GLS 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 GLS content, sometimes showed inconsistent or conflicting results between different studies (López-Berenguer et al., 2007; Rungapamestry et al., 2007; Song and Thornalley, 2007; Volden et al., 2008a; Yuan et al., 2009; Francisco et al., 2010; Pellegrini et al., 2010).

Processing changes GLS content through several mechanisms, such as enzyme-catalyzed 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 GLS content. Using the underlying mechanisms that are critical for a processing method can be a valuable approach to understand the kinetics of GLS changes. In this paper, the health-related properties of GLSs 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 GLS content in *Brassica* vegetables currently published and analyses these changes of GLSs 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 GLSs. Furthermore, the value of a mechanistic approach in future research on process-induced changes in GLSs 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; 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 GLS 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 GLS 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 tu-

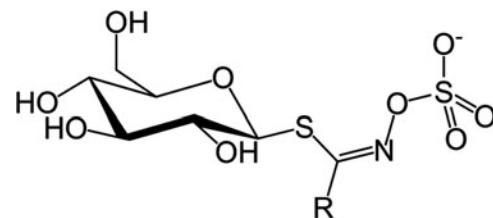


Figure 1 General structure of GLSs (*R* is the variable side chain).

mor initiation, promotion, and progression, and by suppressing the final steps of carcinogenesis (Traka and Mithen, 2009).

GLSs are a group of water-soluble secondary plant metabolites. Structurally, a GLS comprises  $\beta$ -thioglucoside N-hydroxysulphates with a sulphur-linked  $\beta$ -D-glucopyranose moiety and side-chain group (*R*) (Figure 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 GLS can be classified either as aliphatic, aromatic, or indole (Mithen et al., 2000; Verkerk et al., 2009).

GLSs 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, GLSs are highly prone to hydrolytic degradation catalyzed 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 GLS substrate, pH, presence of  $Fe^{2+}$ , or epithiospecifier protein (Mithen et al., 2000; Fahey et al., 2001). Moreover, GLSs 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 GLS 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 gastrointestinal tract. Ingestion of GLSs containing 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 GLS 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 GLS 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 GLS 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 GLSs 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).

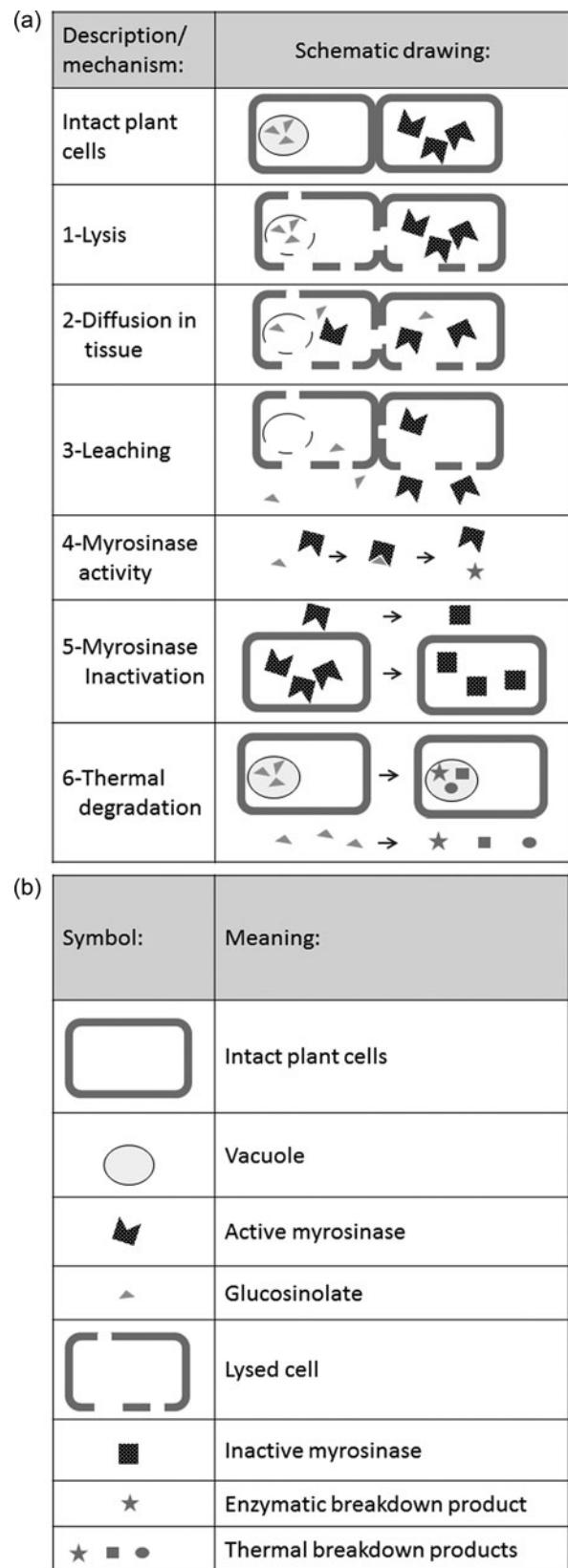
### MECHANISMS UNDERLYING GLUCOSINOLATE CHANGES DURING PROCESSING

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 GLS 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 GLS 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 GLS 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 color 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 GLS 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 GLS 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 and Table 1), namely:

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



**Figure 2** **a.** Schematic illustration of the main mechanisms responsible for the changes in GLS content during *Brassica* vegetable preparation **b.** Legend to explain the used symbols.

**Table 1** Relative importance of the main mechanisms involved in changing the GLS 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	+/-	+/-	+/-	+/-	+

6. leaching of GLSs and breakdown products into boiling water after lysis and diffusion.

The changes in GLS 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 GLS (Oerlemans et al., 2006). Indole GLSs generally react faster than aliphatic GLSs. The variety of the *Brassica* vegetables, moreover, also affects the thermal degradation rate constant of each GLS. So, the reaction rate is depending both on the type of GLS and also on the plant matrix that it is present in (Dekker et al., 2009; Hanschen et al., 2012).

### 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 of 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 GLSs and the myrosinase enzyme will diffuse through the lysed tissue and also partly leach into the cooking water. Enzymatic breakdown of GLSs 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 (nonenzymatic) degradation of GLSs can occur

at boiling temperature. Depending on the process conditions, the type of GLS, and the type of *Brassica* vegetable losses of 5–20% of GLSs 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 GLS 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 GLSs due to leaching are typically expected for boiling processes, depending on the process conditions, the type of GLS, and the type of *Brassica* vegetable. In Table 2, the published results on GLS retention upon boiling are shown. Rungapamestry et al. (2008) reported a small loss, yet statistically significant, of total GLSs 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 GLS content in broccoli and Brussels sprouts but reduced total GLS content in white cauliflowers. They reported, furthermore, a high degree of total GLS 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 GLS content was two-fold higher in boiled cauliflower compared to raw. The authors suspected that this was due to higher extractability of GLSs in the boiled than in the raw cauliflower tissues. Higher degree of GLS 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 GLS contents in turnips greens boiled at either normal or high pressure.

Previous studies showed that the main parts of leached GLSs 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 is likely due to thermal degradation and myrosinase-catalyzed hydrolysis (Rungapamestry et al., 2007; Volden et al., 2009b). Song and Thornalley (2007) found that boiling for 30 minutes retained the sum of GLSs from boiled vegetable and cooking water. However, Ciska and Kozłowska (2001) reported that degree of leaching of GLSs in cabbage during boiling for 5–25 minutes was steady at about 10% of the GLSs 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 GLSs than leaching during boiling of cabbage for 60 min in acidic solution.

To summarize, boiling generally reduce GLS 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

**Table 2** Effect of boiling on GLS retention

<sup>1</sup> Each study observes two or more GLSs. Calculation on totals of aliphatic, indole, and aromatic GLSs is based on published mean value of individual GLSs belonging to the group. Total aromatic is only from chromatographic GLSs. These calculations to biomass are based on *total* *GLS processing methods*.

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N<sup>o</sup> = "not available"

NA = not available.

$\text{NC} =$  not calculated; the fresh or boiled or both treated *Brassicas* cont

due to the effect of heating on the extraction efficiency of GLSs.

### 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 on steaming, in contrast to boiling, steaming either slightly decreases or increases the total or major GLS contents of *Brassica* vegetables (Table 3). Conaway et al. (2000), Vallejo et al. (2002), and Rungapamestry et al. (2006) have reported no significant effect of steaming on total GLS content in cabbage and broccoli with respect to the fresh ones. Song and Thornalley (2007) also reported no significant losses of total GLSs in broccoli, green cabbage, cauliflower, and Brussels sprout over 20 minutes of steaming.

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

As steaming proceeds, myrosinase will finally be inactivated which will lead to a stable GLS 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 minutes but, this showed a loss by 90.4% after 7 minutes.

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 GLS content. Moreover, steaming seems to increase the extractability of the GLSs.

### 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, 2000). 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 GLSs during the core processing. Blanching of *Brassica* vegetable aims to inactivate myrosinase and consequently, can inhibit the GLS enzymatic hydrolysis. Depending on the blanching conditions, the type of GLS, and the type of *Brassica* vegetable lower effects of blanching than boiling are expected on the other mechanisms involved to change GLS content, including cell lysis, diffusion, leaching, and thermal breakdown. On the other hand, blanching in water using high water/vegetable ratio will be favoring leaching upon cell lysis and diffusion.

Blanching was reported to reduce total GLS content quite variable (Table 4), ranging from about 30% through 52% in cauliflowers (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; Watheler et al., 1996; Cieslik et al., 2007). Volden et al. (2008a) reported a great loss at about 64% of total GLSs in red cabbage due to blanching, which was 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 GLS losses of white cabbage and broccoli, respectively, after blanching. Similar to boiling, the main parts of the GLS 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 behavior of GLSs over blanching. Goodrich et al. (1989) have compared total GLS contents in broccoli and Brussels sprouts after hot water and steam blanching techniques. The authors reported no significant losses of total GLS 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 GLS contents in broccoli significantly by 83% and 40%, respectively.

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

**Table 3** Effect of steaming on GLS retention

<i>Brassica</i>	Temperature (°C)	Time (min)	Size	<i>Brassica</i> Weight (g)	Water volume (mL)	GLS Retention (%)			
						Aliphatic	Indole	Aromatic	
Broccoli	100 (oven)	13	Floret + stem 2.5 cm	Nine 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 Gliszczynska-Åwiglo 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	Nine specimens		89.5	131.9		130.8 Miglio et al. (2008)
Boiling water	3.5		Floret 3 cm dim + stem 1 cm	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 (two 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	Nine 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)
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 (five 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	Nine specimens	NA	68.7	137.9	118.0 Pellegrini et al. (2010)	
		12	Floret (initially frozen)						
	Boiling water	11	Floret + stem 2.5 cm			50.4	104.3		97.3
		10	Floret (initially frozen)			89.3	102.3		88.8
Turnip greens	15		NA	150	1500	86.3	98.8	97.6	97.5 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.

**Table 4** Effect of blanching<sup>2</sup> on GLS retention

<i>Brassica</i>	Temperature (°C)	Time (min)	Size	<i>Brassica</i> weight (g)	Water volume (mL)	GLS Retention (%)			References
						Aliphatic	Indole	Aromatic	
Broccoli	80	3	NA	NA	NA	64.7	97.2	NC	69.9 Cieslik et al. (2007)
	99	4	500	500	22.4	11.5	NA	NA	17.0 Goodrich et al. (1989)
	Steam: 99–102	5.5			72.2	47.2			60.1 Cieslik et al. (2007)
Brussels sprouts	80	3	NA	NA	75.5	70.8	79.5	NA	71.9 Goodrich et al. (1989)
	99	4	500	500	105.6	88.3	NA	NA	93.2 Wathelet et al. (1996)
	Steam: 99–102	5.5			74.3	79.4			78.0 Wathelet et al. (1996)
90 or 95; or steam: 105	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)
	94–96	3	1 × 1 cm 1.5 mm (2 cultivars)	300	3000	34.5	33.5	39.1	35.8 Volden et al. (2008a)
	Boiling water	5		2000	2000	25.1 & 60.6	27.2 & 37.0	NA	25.7 & 49.6 Wennerberg 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; 2009b)
	Green Cauliflowers	80	NA	3000	56–69	43–70			48–70 Cieslik et al. (2007)
	White Cauliflowers			NA	89.4	119.7	100.0	97.4 NA	87.3 33.3 78.8
Curly kale					97.7	78.6	80.0	74.7	

<sup>2</sup>water blanching; otherwise is specified.

NA = not available.

NC = not calculated; the fresh or blanched or both treated *Brassicas* contains traces of GLSs.

Several mechanisms, particularly cell lysis, diffusion, and myrosinase inactivation, take place during microwave processing determining the fate of GLS content. Degree of changes of GLS content is strongly affected by processing time and power output. Longer treatment times 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 GLS contents (Table 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 GLSs 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 minutes). Despite of few losses of total GLSs, the authors found that these combinations increased total GLS 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 minutes on cabbage. The authors found the loss of total GLS content in cabbages by 17.3% after microwave processing for 7 minutes. Accordingly, López-Berenguer et al. (2007) reported general decrease of total GLSs in broccoli which was added with 150 mL of water after microwave processing for 5 minutes 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 seconds of microwave processing at 750 W and further decreased by 96.7% after 2 minutes.

Leaching of GLSs 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 minutes at 1000 W caused a great loss of total GLS content of broccoli florets by about 74% but, the recovery of total GLSs was only about 1% in water. This was expected due to loss of cooking water containing leached GLSs (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 break-

down. A similar microwave processing condition on broccoli, however, reduced about 18% of total GLS 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 GLSs in broccoli by about 60% after microwave processing for 5 minutes 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 GLS 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 GLSs.

### **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 vaporized (Fellows, 2000). 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 can retain the total GLSs and most of individual GLS 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 GLS loss. However, Yuan et al. (2009) observed stir-frying of broccoli reduced considerable amount of the aliphatic and indole GLSs by about 55% and 67%, respectively, possibly due to extensive time-temperature employed (Table 6).

During stir-frying, thermal degradation mechanism might involve predominantly than leaching to degrade GLS 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 GLS losses between these techniques. When deep frying was applied, total GLSs 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 GLS content particularly for aliphatic and aromatic GLSs (Rungapamestry et al., 2008).

**Table 5** Effect of microwave processing on GLS retention

<i>Brassica</i>	Power (W)	Time (min)	Size	<i>Brassica</i> weight (g)	Water Addition (mL)	GLS Retention (%)				
						Aliphatic	Indole	Aromatic		
Broccoli	300	30	Floret + stem 2.5 cm	10 specimens	—	97.7 70.8	110.4 75.0	NA	102.2 73.4	Pellegrini et al. (2010)
	13		Floret + stem (initially frozen)	—						
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	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	25.5	Vallejo et al. (2002)
			Piece	200	10	39.7	46.9	NA	41.8	Yuan et al. (2009)
			Floret	20–30	10% of solid	NA	NA	NA	NS loss	Song and Thornalley (2007)
Brussels sprout	900	0.5–3	Whole (initially frozen)	10 specimens	—	86.7	148.6	—	120.3	Pellegrini et al. (2010)
			Whole	—	10% of solid	64.7	99.8	—	80.9	Song and Thornalley (2007)
				20–30	10% of solid	NA	NA	NA	NS loss	
Cabbage			Whole or largely cut leaves		10% of solid					
Red cabbage	180; 540; or 900	36 seconds–24 minutes	1 cm	300	—	77.5–189.6	92.9–175.8	88.0–178.0	88.0–178.0	Verkerk and Dekker (2004)
Cauliflower	900	0.5–3	Floret	20–30	10% of solid	NA	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	72.4	Pellegrini et al. (2010)
		20	Floret (initially frozen)	—	107.1	117.6	117.6	113.8	113.8	

NC = not calculated; the fresh or microwaved or both treated *Brassicas* contains traces of GLSs.

NA = not available.

NS = not significant.

**Table 6** Effect of stir-frying on GLS retention

<i>Brassica</i>	Oil temperature (°C)	Time (min)	Size	<i>Brassica</i> weight (g)	Oil volume (mL)	Water addition (mL)	GLS Retention (%)			
							Aliphatic	Indole	Aromatic	
Broccoli	Hot olive oil Broccoli temp.: 80	2 + 2*	Floret 40 mm length	110	5	7	99.6	98.5	0.5	98.7
	125–140	3.5	Floret 3 cm Piece	150	40	—	NA	NA	NA	Rungapamestry et al. (2008)
	130–140	5	—	200	10	—	45.0	36.2	41.6	Moreno et al. (2007)
	Pre-heated to 200; kept at 110–120	2 + 3*	Strips 1 cm	20–30	15% w/w	NA	50	42.4	43.4	Yuan et al. (2009)
		3 & 5				NA	NA	NA	NS loss	Song and Thornalley (2007)
Brussels sprout										
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.

The types of cooking oil used for stir-frying might also influence on the fate of GLS content. Stir-frying using refined olive and sunflower oils reduced total GLS 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 GLS 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 GLS contents.

To conclude, limited or no leaching, enzymatic hydrolysis, and thermal degradation occur during short stir frying slightly reduce the GLS 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 GLSs.

### Fermentation

Fermentation is one among the old processing methods and have formed a traditional part of the diet in many countries (Fellows, 2000). The fermented *Brassica* vegetable commonly studied, particularly in European region, is sauerkraut (de Vos and Blijlevens, 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 GLS 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 GLSs 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 GLS content substantially. No GLS 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-MeO-glucobrassicin in the final fermented cabbage in small quantities. Meanwhile, Suzuki et al. (2006) reported that the GLSs in *nozawana-zuke*, a fermented product of *Brassica rapa* L., were glucobrassicin and gluconasturtiin as the major GLSs, and gluconapin, 4-OH-glucobrassicin, glucoerteroin, and 4-MeO-glucobrassicin in small quantities relative to the fresh *nozawana*.

Ciska and Pathak (2004) detected the breakdown products of the GLSs in sauerkraut, included ITCs and cyanides from aliphatic GLSs, 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 GLS 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 GLS 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 did not depend only on the content of native GLSs in raw cabbage but, it might also substantially depend 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 behavior of individual GLSs during fermentation. The authors reported that the GLS content decreased after seven days of fermentation but, the ratio of indole GLSs to total GLSs was much higher than before fermentation. It was proposed that a stress response under salting treatment and compression and/or myrosinase resistance were the possible factors. In addition, they found that a shorter maturation period retained more GLS content. Leaching, meanwhile, hardly involve in the fermentation as most GLSs was retained in the tissues and no GLSs were detected in the brine (Suzuki et al., 2006).

To summarize, fermentation can reduce the amounts of GLSs 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 GLS degradation.

### Other Processing Methods

The effects of freezing, drying, and pressure/temperature processing have also been studied on the GLS 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 GLS content of *Brassica* vegetables can be retained during freezing and frozen storage. Rungapamestry et al. (2008) reported that freezing the blanched broccoli at  $-18^{\circ}\text{C}$  within 20 minutes by using air blast freezer did not substantially change the total GLS content. The contents of glucoiberin, glucoraphanin, and gluconasturtiin were retained and glucopoleiferin content was increased by 50% but glucobrassicin

and 4-MeO-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  $-20^{\circ}\text{C}$  retained the total GLS content in the principal inflorescences but significantly decreased the total GLS content in the secondary inflorescences. The authors suspected that the loose structure of the broccoli stalk and flower head were very susceptible to the leaching effects during prior blanching.

During storage of blanch-frozen broccoli at  $-20^{\circ}\text{C}$  for 90 days, the GLS 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  $-24^{\circ}\text{C}$  for 12 months on the total GLS content. Significant losses of GLS contents were found on specific cultivars after three months of storage. The individual GLSs, 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  $-22^{\circ}\text{C}$  for 48 hours did not produce any consistent changes in total GLS content. Losses of total GLS contents relative to the blanched vegetables were 50.7% in frozen Brussels sprouts and 4.5% in frozen curly kale. In contrast, the total GLS 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  $-85^{\circ}\text{C}$  for two months without prior blanching caused significant loss of GLSs. The authors suspected that this was due to freeze-thaw fracture of plant cells and accessibility of myrosinase to GLSs during thawing. Freezing ruptured plant cells and softened vegetables because of water crystallization in extracellular and intracellular spaces within the vegetable matrix.

The effects of drying conditions, namely temperature (at 50 through  $100^{\circ}\text{C}$ ) and drying air velocity (at 1.2 through  $2.25\text{ ms}^{-1}$ ) on the content of indole GLSs in broccoli were examined (Mrkic et al., 2010). The authors found that different drying conditions affected individual GLSs differently. The remaining individual GLSs of the dried relative to the blanched broccoli were in the range of about 32–90% for 4-OH-glucobrassicin, 65–92% for glucobrassicin, 29–90% for 4-MeO-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 GLS 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 could bring an active form of myrosinase into contact with the GLSs and consequently, improve the health beneficial compounds production (Van Eijken et al., 2009). Van Eijken et al. (2009) reported that GLS hydrolysis could be induced during mild pressure processing of broccoli. About 20% loss of GLSs was observed after 35 minutes of treatment at  $20^{\circ}\text{C}$  and elevated pressure (200–300 MPa). Meanwhile, the GLS degradation was found after 15 minutes of treatment at

$40^{\circ}\text{C}$  and 100–500 MPa and the highest lost, i.e. 63% of GLSs, was observed at 300 MPa. Furthermore, the hydrolysis products of the aliphatic GLSs 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 GLS 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 (PEF), cold plasma, and advanced heating technologies including microwaves, ohmic- and radio frequency heating. Possible effects of these new technologies on GLSs 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 GLS behavior 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 GLSs.

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 tastes and textures 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*, *dakquadong*, *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 methods 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 choy-sum (*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 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 and the whole day for 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 GLS content in *Brassica* vegetables. Some of the methods might reduce the GLS content significantly while others might retain most of their content. To our knowledge, these specific preparation methods are scarcely studied. Therefore, a study on the effect 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 GLSs diversely, depending on the processing method and conditions, the type of *Brassica* vegetable, and the type of GLSs. A mechanism approach underlying each processing method could explain the behavior of GLS contents. Boiling and blanching can reduce the GLS content considerably due to rigorous mechanisms of cell lysis, diffusion, thermal degradation, and leaching. Fermentation also reduces GLS content of *Brassica* vegetables; however, more mechanistic-based processing studies should be further performed to reveal breakdown mechanisms of GLS content thoroughly. Steaming and microwave processing relatively retain or even increase total GLS content because of low impact on mechanisms of cell lysis, diffusion, thermal degradation, and leaching but can increase the extractability of GLSs in plant matrix. Stir-frying may slightly reduce GLS content in a lower level than boiling and blanching. Meanwhile, changes of individual GLSs are more intricate to be explained, involving individual

GLS 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 behavior of GLS content under processed. Studies on various preparation methods from other regions and culture will also enrich the understanding of the GLS behavior in specific conditions. In addition, optimizing GLS 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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