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Glucosinolates

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Glucosinolates

With 83 Figures and 23 Tables

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Preface

Glucosinolates, natural S-glycosides, have attained importance in recent years as new class of secondary metabolites of profound physiological properties. Glucosinolates are present in the 16 families of order Brassicales including Brassicaceae which contains several of daily vegetables (cabbage, radish, mustard, cauliflower, broccoli, horseradish, turnip, oilseed rape, etc.). Glucosinolates are accumulated in all plant parts such as root, shoot, stem, and seed and also contain an enzyme called myrosinase (b-thioglucosidase). Glucosinolates have become important parameter to breed and develop new crop varieties for human welfare. They possess wide ranging properties like bacteriocide, antioxidant, bioherbicide and fungicide, and anticarcinogenic; therefore, this book is a timely compilation of state of information about this rapidly developing field.

The book aims to present comprehensive and up-to-date information on this new and developing field. The book comprises of 15 chapters and is divided into three sections, viz.: Part I – Biology, Phytochemistry, Genetics, and Defense; Part II – Biological Activity; and Part III – Analytical and Processing Methods. This comprehensive reference book presents the sources of glucosinolates, genetics and breeding of *Brassica* crops, glucosinolates in food, glucosinolates in plant defense, antimicrobial activity, neuroprotective effects, glucosinolates in atherosclerosis, anticancerous effect and as modulator of drugs, methods of glucosinolates extraction, preparation, processing, and identification by mass spectroscopy. The book will be a valuable source on glucosinolates.

The book is intended to serve the needs of graduate students, scholars, and researchers in the field of botany, agriculture, pharmacy, biotechnology, and phytochemistry; industrial scientists; and those involved in processing and marketing of vegetable products.

This work could not be completed without active support of Springer team who took pains in streamlining the production process. We are particularly indebted to Drs. Lydia Mueller, Sylvia Blago, and Sylvia Jakuscheit for their continuous professional support throughout the project.

January 2017

J.-M. Mérillon
K.G. Ramawat
Editors

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About the Editors

Prof. Dr. Jean-Michel Mérillon received his Pharm.D. (1979), Ph.D. (1984), and HDR (1992) from the University of Tours in France. He joined this same university as assistant professor in 1981 and became associate professor in 1987. In 1993, he moved to the faculty of Pharmacy, University of Bordeaux, France, accepting a position as full professor. He is currently leading the "study group on biologically active plant substances" at the Institute of Vine and Wine Sciences, which comprises 25 scientists and research students. The group has been working on phenolic compounds from vine and wine for many years, mainly complex stilbenes and their involvement in health. Prof. Mérillon has supervised the doctoral theses of 20 students. He is involved in developing teaching on plantbiology, natural bioactive compounds, and biotechnology.

Prof. Mérillon has published more than 150 research papers in internationally recognized journals, resulting in an H index of 38 (documents published between 1996 and 2016). He has coedited books and reference works on secondary metabolites and biotechnology.

Throughout his career, Prof. Mérillon has traveled widely as a senior professor. Scientists from several countries have been and are working in his laboratory, and his research is supported by funding from the Aquitaine Regional Government, the Ministry of Higher Education and Research, and various private companies. In 2004, he founded the technology transfer unit "Polyphenols Biotech," providing support for R&D programs for SMEs and major groups from the cosmetic, pharmaceutical, agricultural, and health-nutrition sectors. Faculty of Pharmacy, Institut des Sciences de la Vigne et du Vin – CS 50008, University of Bordeaux, Villenave d'Ornon, France.

Prof. Dr. Kishan Gopal Ramawat is former professor and head of the Botany Department, M.L. Sukhadia University, Udaipur, India, and can look back on longstanding research experience. He received his Ph.D. in Plant Biotechnology in 1978 from the University of Jodhpur, India, and afterwards joined the university as a faculty member. In 1991, he moved to the M.L. Sukhadia University in Udaipur as associate professor and became professor in 2001. He served as the head of the Department of Botany (2001–2004, 2010–2012); was in charge of the Department of Biotechnology (2003–2004); was a member of the task force on medicinal and aromatic plants of the Department of Biotechnology, Government of India, New Delhi (2002–2005); and coordinated UGC-DRS and DST-FIST programs (2002–2012).

Prof. Ramawat had done his postdoctoral studies at the University of Tours, France, from 1983 to 1985, and later returned to Tours as visiting professor (1991). He also visited the University of Bordeaux 2, France, several times as visiting professor (1995, 1999, 2003, 2006, 2010) and in 2005 Poland in an academic exchange program (2005). Through these visits in France, Prof. Ramawat and Prof. Mérillon established a strong connection, which has resulted in productive collaborations and several book and reference work publications.

Prof. Ramawat has published more than 170 well-cited peer-reviewed papers and articles and edited several books and reference works on topics such as the biotechnology of medicinal plants, secondary metabolites, bioactive molecules, herbal drugs, and many other topics. His research was funded by several funding agencies.

In his research group, Prof. Ramawat has supervised doctoral theses of 25 students. He is an active member of several academic bodies, associations, and editorial boards of journals. Botany Department, M.L.Sukhadia University, Udaipur, India.

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Processing and Preparation of *Brassica* Vegetables and the Fate of Glucosinolates

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Probo Yulianto Nugrahedi, Matthijs Dekker, and Ruud Verkerk

Abstract

The healthiness of a vegetable cannot solely be inferred from the amount of health-promoting compounds in the raw materials. *Brassica* vegetables, for example, are consumed mostly after processing to improve palatability and to extend the shelf life. However, processing also results to various changes in the content of glucosinolates which intakes are associated with a reduced risk of several cancers. The large variety in cooking practices and processing methods affect the glucosinolate content in the vegetables, particularly due to processes that allow for enzymatic hydrolysis and thermal degradation of glucosinolates, and leaching of the bioactive components. Knowledge on the effect of preparation and processing of *Brassica* vegetables is important to evaluate the healthiness of the consumed product and to investigate mechanisms to retain high glucosinolate levels at the stage of consumption and to increase the intake of health-protective compounds by the consumer. By using a mechanistic approach, the fate of glucosinolates during different processing and preparation methods and conditions can be explained. Boiling and blanching reduce the glucosinolate content significantly particularly because of the mechanisms of leaching following cell lysis and diffusion, and partly due to thermal and enzymatic degradation. Steaming, microwave processing, and stir frying either retain or only slightly reduce the glucosinolate content due to low degrees of leaching. These methods

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can enhance the accessibility of glucosinolates from the plant tissue. Fermentation reduces the glucosinolate content considerably, the underlying mechanisms are not yet completely clear, but enzymatic breakdown seems to play an important role. Studying the changes of glucosinolates during processing by a mechanistic approach is shown to be valuable to redesign the processing and to reformulate the product for improving health benefits of these compounds.

Keywords
Glucosinolate • Preparation • Processing • Mechanistic approach • *Brassica* vegetable

Abbreviations

ESP	Epithiospecifier protein
GS	Glucosinolate
HPP	High pressure processing
ITC	Isothiocyanate
MW	Microwave

Contents

1 Introduction

Many representatives of the Brassicaceae family are of particular importance as vegetables for our human diet, such as *Brassica oleracea* (e.g., cabbage, cauliflower, broccoli, and Brussels sprouts), *Brassica rapa* (Chinese cabbage, pak choi, and turnip), *Brassica juncea* (Indian and Chinese mustard), *Brassica napus* (rutabaga and swede), and as seasonings and relishes (e.g., mustard and wasabi). *Brassica* vegetables are unique in that they are rich sources of glucosinolates (GSs), sulfur-containing compounds that deliver a pungent aroma and spicy or bitter taste [1–3]. Moreover, GSs are claimed to be bioactive components responsible for many of the physiological health effects proposed for *Brassica* vegetables in different types of studies, including in vitro, animal, human, and epidemiological studies [4, 5].

Most of the vegetables need some kind of treatment, either household preparation or industrial processing, in order to make them suitable and palatable for consumption. The large variety in cooking and processing methods and conditions have in common that they all affect the profile of GSs in the vegetables, particularly due to (bio)chemical and physical processes such as enzymatic hydrolysis and thermal degradation of GSs, and leaching of the bioactive components out of the plant tissue [6]. As a consequence, we can say that the healthiness of a vegetable cannot solely be inferred from the amount of the nutritional and health compounds in the raw materials. Knowledge on the effect of preparation and processing of *Brassica* vegetables is important to evaluate the healthiness of the final consumed product and to investigate ways to retain high GS levels at the stage of consumption and to increase the intake of health-protective compounds by the consumer.

2 *Brassica* Vegetable Intake and Health

Brassica vegetables, like broccoli, cauliflower, Brussels sprouts, and red cabbage, contain significant levels of health-promoting constituents, including vitamins, minerals, fibers, and numerous types of secondary plant metabolites also called phytochemicals. Many phytochemicals from vegetables contribute to the reported antioxidative, anti-inflammatory, anticarcinogenic, and cardiovascular protective effects [7]. One group of phytochemicals occurring almost exclusively in *Brassica* vegetables is the group of glucosinolates [8]. Epidemiological studies showed that the intake of *Brassica* vegetables is inversely associated with the risk of certain types of cancer, including colorectal and lung cancers [9–13].

Glucosinolates (GSs) intake is expected to play a significant role in lowering this risk of cancer. However, in epidemiologic studies, the intake of the vegetables is monitored, the real intake of protective compounds, like GSs, is often an unknown variable. It is demonstrated that many steps in the food production chain, like cultivation, storage, processing, and preparation of vegetables can dramatically affect the content and thus the intake of phytochemicals such as GSs in *Brassica* vegetables [14].

3 Glucosinolates in *Brassica* vegetables

3.1 Occurrence

Glucosinolates (GSs), a group of plant secondary metabolites, contain β-thioglucoside *N*-hydroxysulfates with a sulfur linked β-D-glucopyranose moiety and variable side group (*R*), which usually classifies the aliphatic, aromatic, and indole GSs. These three GS groups are frequently found in *Brassica* vegetable species (Table 1). Moreover, each species of the family Brassicaceae has a distinct GS profile characterized by major GSs as reviewed by Verkerk et al. [5]. Also, different species of the same genus and different cultivars of the same species can

have highly variable GS concentrations. The majority of GSs are found in every plant organ although the concentration and composition of the GSs can vary greatly and can also change during plant development [5]. Usually, a single plant species contains up to four different GSs in significant amounts, while as many as 15 different GSs can be found in lower amounts in the same plant. *Brassica* vegetables occur in different appearances: leafy (e.g., collard green and rocket salad), flowering (cauliflower and broccoli), stems (kohlrabi), roots (radish, rutabaga, and turnips), and buds (Brussels sprouts and cabbage). The content of GSs varies in these different tissues, for example, GS concentrations are higher in the florets than in the stalks of broccoli [5]. The seeds and the sprouting vegetables or cresses, such as garden cress or watercress, usually contain one specific type of GS in substantial amounts. The GS concentrations in vegetables, although often highly variable, are around 1% dry weight in some *Brassica* vegetables.

3.2 Glucosinolate/Myrosinase System

The special feature of GS-containing vegetables is the system of compartmentalization of GSs and the presence of specialized myrosin cells containing the hydrolytic

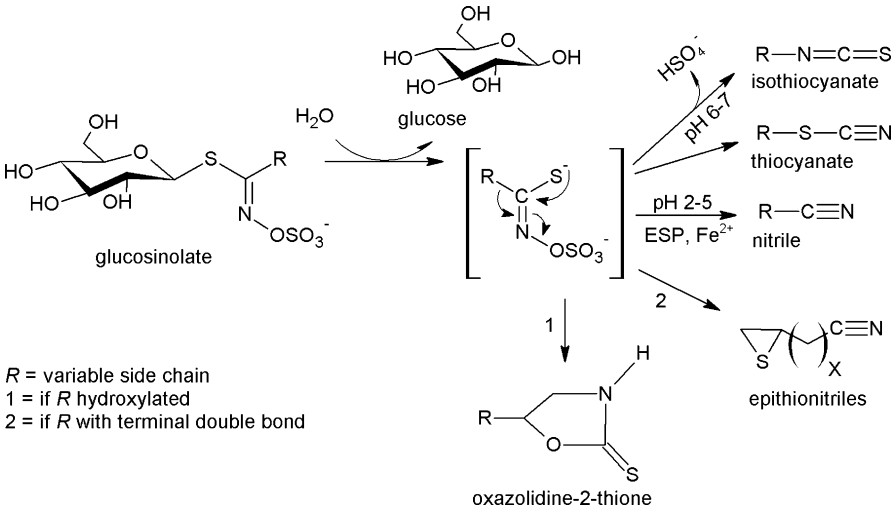
Table 1 Glucosinolates commonly found in *Brassica* vegetables [15]

Trivial name	Chemical name	Main source
Aliphatic		
Glucoibervirin	3-Methylthiopropyl-GS	Green and white cauliflowers
Glucoerucin	4-Methylthiobutyl-GS	Rocket
Glucoiberin	3-Methylsulfinylpropyl-GS	Broccoli sprouts, Savoy cabbage
Glucoraphanin	4-Methylsulfinylbutyl-GS	Broccoli(cress), Red cabbage
Sinigrin	Prop-2-enyl-GS	Brussels sprouts, White cauliflower
Gluconapin	But-3-enyl-GS	Pak choi
Glucobrassicanapin	Pent-4-enyl-GS	Chinese cabbage, Pak choi
Progoitrin	(*2R*)-2-Hydroxybut-3-enyl	Turnip, Chinese broccoli
Indole		
Glucobrassicin	Indol-3-ylmethyl-GS	Broccoli, Cauliflower, and many more
4-Hydroxy-glucobrassicin	4-Hydroxy-indol-3-ylmethyl-GS	Broccoli, Cauliflower, and many more
4-Methoxy-glucobrassicin	4-Methoxy-indol-3-ylmethyl-GS	Broccoli, Cauliflower, and many more
Neo-glucobrassicin	N-methoxyindol-3-ylmethyl-GS	Broccoli, Cauliflower, and many more
Aromatic		
Glucotropaeolin	Benzyl-GS	Garden cress
Gluconasturtiin	Phenylethyl-GS	Water cress

enzyme myrosinase (thioglucoside glucohydrolase, EC 3.2.1.147). Upon plant's cell disruption, GSs are highly prone to degradation by myrosinase-catalyzed hydrolysis (see Scheme 1). Subsequently, the GS will degrade into glucose and an unstable aglycon intermediate. The unstable aglycon rearranges into different breakdown products, including isothiocyanates (ITCs), thiocyanates, nitriles, and epithionitriles, depending on conditions described in the Sect. 3.3 [4, 8].

Myrosinase is a relative thermo labile enzyme, which can be readily denatured at moderate to high temperatures. Especially temperatures applied during processing of *Brassica* vegetables quickly inactivate myrosinase [16–19]. The optimum temperatures for the activity of myrosinase are different between *Brassica* vegetables, in the range between 30 °C and 60 °C, and the activity is also influenced by pH, the presence of ascorbic acid, salt, and pressure [20–24]. During the various stages of storage, preparation, cooking, and processing of the vegetables, the GS–myrosinase system is affected in a complex way. Dekker et al. [25] have estimated that the concentration of GSs in *Brassica* vegetables may vary by five to tenfold at each stage.

The health-promoting effect of GSs is mainly attributed to the ITCs that are formed due to hydrolysis by myrosinase after tissue damage. Since myrosinase is mostly inactivated during processing or preparation, formation of ITCs usually does not occur in the product during mastication. However, a myrosinase-like activity is also provided by the microflora in the human's large intestine. Intake of *Brassica* products containing inactive endogenous plant myrosinase still can have benefit by the formation and absorption of bioactive breakdown products by enzymes from the gut flora. However, their bioavailability is lower than the ones with active plant myrosinase [10, 26–28].



Scheme 1 Enzymatic breakdown of glucosinolate [adapted from 15]

3.3 Breakdown Products of Glucosinolates

Although GSs can be chemically degraded at higher temperatures [29–31], the hydrolysis is mainly enzymatically driven. Previous reports have reviewed the mechanisms of GS hydrolysis [e.g., 4, 31–34], which will be briefly described here. Several products of hydrolysis of a GS can be produced, such as ITCs, thiocyanates, nitriles, epithionitriles, oxazolidine-2-thiones, or indole compounds, depending on the structure of the GS side chain, the reaction conditions (e.g., pH), presence of additional cofactors (e.g., Fe^{2+}), and proteins (e.g., epithiospecifier protein (ESP) and thiocyanate-forming protein). Most frequently, the aglycon undergoes a Lossen arrangement to produce an ITC.

At neutral pH, the major hydrolysis products are stable ITCs. For example, hydrolysis of gluconapin and sinigrin produces mainly ITCs, namely, 3-butenyl-ITC and 2-propenyl-ITC, respectively. Sulforaphane, the ITC derived from glucoraphanin, is the most widely studied as the most bioactive GS hydrolysis product. For GSs having a β-hydroxylated side chain or an indole moiety, β-hydroxy-ITCs are unstable and spontaneously cyclize to oxazolidine-2-thiones, while indole ITCs undergo breakdown producing, for example, indole-3-carbinol (I3C).

At low pH, in the presence of an ESP and ferrous ions, gluconapin and sinigrin produce cyano-epithioalkane, such as 1-cyano-3,4-epithiobutane and 1-cyano-2,3-epithiopropane, respectively, and progoitrin is hydrolyzed into an epithionitriles. Nitriles are the major degradation products under acidic conditions, which can be diminished by heating. It is formed after hydrolysis of a GS with a side chain lacking a double bond, which may involve ESP. Conversion to nitriles is also enhanced in the presence of ferrous ions. Indole GSs can form indolyl-3-acetonitrile and elemental sulfur. Moreover, ascorbigen and thiocyanate are the major products of indole GSs between pH 4 and 7 in the presence of ascorbic acid. For thiocyanates production, the mechanism from GSs is not clear yet.

4 Glucosinolate During Preparation of *Brassica* Vegetables

Brassica vegetables are mainly consumed after processing, either at a domestic or industrial level. Broccoli, cauliflower, and cabbage are boiled, steamed, stir-fried, or microwave-processed during domestic preparation to produce various dishes. Canned or fermented vegetables are also produced after industrial processing of these vegetables. Even when consumed raw, for example, in a salad, these vegetables are firstly prepared, namely, by washing, cutting, and chopping. Various products or dishes available around the globe are made based on these vegetables. For examples, many kinds of soup, steamed, and stir-fried *Brassica* produced by domestic preparation and industrial processed products, such as canned and fermented *Brassica* such as sauerkraut, or more local products as *sayur asin*, and *kimchi*. In some Asian countries, the dishes made from these vegetables are usually considered as a side dish to accompany rice or noodle [35].

Postharvest treatments of *Brassica* vegetables, such as cutting or chopping and packaging and storage, can reduce the GS content to a lower degree than the loss due to preparation itself [5, 36–38]. However, a study on storage of chopped cabbage and broccoli was reported to increase the indole GSs, which is suspected as physiological response similar to response due to insect attack [39].

The preparation methods are varied depending on the types of the vegetables, the quality attributes of intended products, and the local customs, particularly for processing at domestic level. At industrial processing level, these are more manageable and standardized. In the Southeast Asian cuisine, for example, cooking vegetables also commonly involves the addition of spices, garlic, chili, salt, sugar, etc., as ingredients for getting the optimum sensorial quality.

Preparation of vegetables is performed to increase the palatability and digestibility, change the sensorial properties (including softening of the texture, improving the appearance and taste), and minimize the risk of microbial contamination. Despite these advantages, preparation can considerably reduce the content of nutrients and phytochemicals in the vegetables including the GS, polyphenols, and ascorbic acid [40–44].

The changes of GSs due to preparation not always have negative implications to health. Although preparation can reduce the GS content, it can at the same time increase the GS accessibility of the product [45]. Depending on the decrease of the content and the increase of the accessibility, the eventual availability of GS for conversion and absorption during digestion can actually be improved by proper preparation.

4.1 Mechanisms Underlying the GS Changes

Previous studies reported a variety of results on the effects of preparation methods on GS content in *Brassica* vegetables. These lead to the complexity to interpret data directly due to the large variability of processing conditions and analytical methods that were used in the various studies. Therefore, a mechanistic approach was proposed to discuss these data by identifying the relative importance of underlying mechanisms affecting GS changes of each preparation method [6, 25].

Either sequential or simultaneous mechanisms take place during preparation (Fig. 1), depending on the processing conditions such as the temperature-time profile. These can involve physical, (bio)chemical reactions, heat, and mass transfer. These different mechanisms can be described as follows:

1. Lysis of cells and cellular compartments
   Cutting or chopping is applied prior to preparation of *Brassica* vegetable. Consequently, vegetable tissue, cells, and cellular compartments are broken. This cell lysis continues during preparation, particularly when heat is applied. During heating of the vegetable, lysis of the cell will gradually occur. Cell and cell organelle membranes will collapse and cell walls will soften. The method of processing and the type of the vegetable determine the degree of lysis.

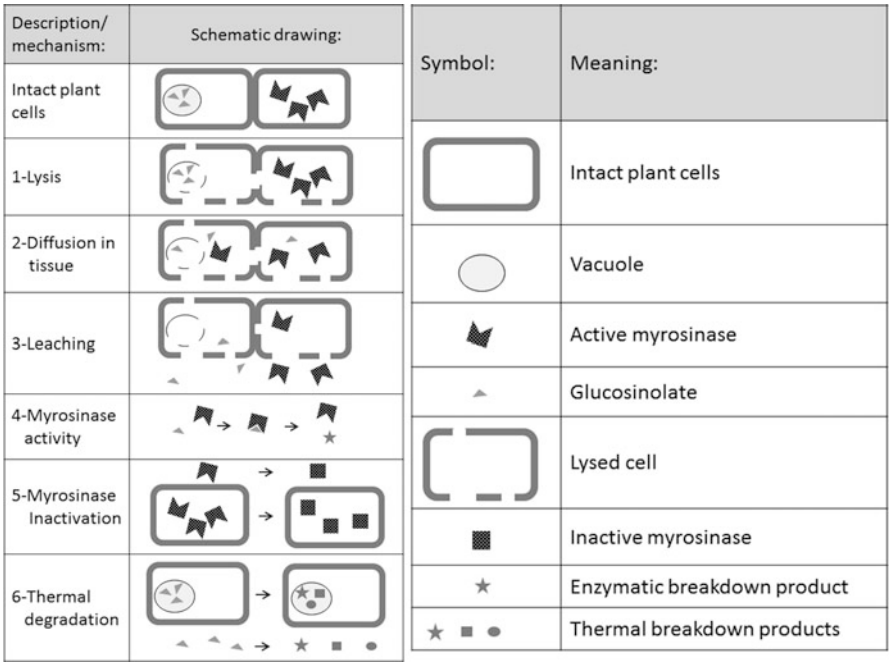


Fig. 1 *Left*: Schematic illustration of the main mechanisms responsible for the changes in glucosinolate content during *Brassica* vegetable preparation. *Right*: Legend to explain the used symbols (Taken from Nugrahedi et al. [6])

2. Diffusion of components through the lysed tissue
   Due to the disruption, components of the cells and cellular compartments, including GSs and myrosinase, will diffuse giving the opportunity for (bio) chemical reactions between these components.
3. Myrosinase-catalyzed hydrolysis of GSs
   Upon lysis and diffusion, myrosinase can have a contact to GSs and the hydrolysis of GSs occurs. The hydrolysis reaction can happen in the lysed tissue and in the cooking water, when the preparation involves water.
4. Thermal degradation of GSs
   Most preparation methods on *Brassica* vegetables apply heat. This is transferred into the plant tissue, for example, by water, steam, or cooking oil. Consequently, GSs can be chemically degraded due to the elevated temperature.
5. Inactivation of myrosinase
   Heat treatment can also cause inactivation of myrosinase, as well as inactivation of the ESP and the thiocyanate-forming protein. Also a loss can occur in the enzymatic cofactors for myrosinase, such as ascorbic acid and Fe^{2+} affecting the outcome of the hydrolysis.
6. Leaching of GSs and breakdown products

When preparation of vegetable involves water, for example, for boiling, GSs as well as the breakdown products will leach into the water, following lysis and diffusion.

To identify the underlying mechanisms involved in each preparation method, all details of preparation conditions, for example, time, temperature, size or weight of *Brassica* vegetables, and water to vegetable ratio, must be taken into account. Hence, the underlying mechanisms of GS changes in each preparation method are specific. For instance, cell lysis, diffusion in tissue, and myrosinase inactivation are identified as the main mechanisms in all preparation methods involving heat. Leaching is identified as the main mechanism affecting GS losses in boiling vegetables, but not for stir frying, short-term steaming, or microwave processing without additional water. Meanwhile, thermal degradation of GS is one of the main mechanisms involved in stir-frying, but also for other preparation methods involving heat this could play an important role depending on the conditions, such as the method (hot vs. cold start boiling), temperature, time, and the size of the vegetable parts. In Sects. 4.2 and 4.3, examples of various conditions between preparation methods will be described further.

Another benefit of using the mechanistic approach is that the GS content and all factors/conditions involved in the preparation can be predicted and optimized quantitatively by applying kinetic modeling. It is a tool to understand what is happening since the proposed mechanisms need to be confronted with experiments. These mechanisms can be subsequently formulated into mathematical equations describing the rate constant of each mechanism. The reaction rate depends on the type of GS and the plant matrix. For more detailed information, previous reports have been studied mathematical modeling of GS changes during preparation of *Brassica* vegetables [46–49].

4.2 Thermal Processing and Preparation Methods

Brassica vegetables are mainly cooked by employing high temperature. Heat treatment affects the changes of GS content mainly by the mechanisms of cell lysis and diffusion followed by thermal degradation of GSs and myrosinase inactivation. Depending on the processing conditions, such as temperature and water to vegetable ratio, other mechanisms can also play an important role, including enzymatic hydrolysis of GSs and leaching.

4.2.1 Boiling, Steaming, Blanching, and Canning

Brassica vegetables are commonly prepared either by boiling or steaming. For a longer preservative effect, canning can also be employed. Meanwhile, blanching is considered as a pretreatment prior to the core processing. In daily practice, blanching is sometimes considered as a light boiling without further cooling. Boiling is performed by immersing the vegetable into cold or already boiling water. Meanwhile, steaming is employed by exposing the vegetable to saturated steam. During

boiling, heat is transferred mainly by convection of hot water into the vegetable tissue. While for steaming, heat is transferred mainly by condensation of steam at the vegetable surface and by convection.

During boiling or water blanching, the heat of water transferred into the vegetable tissue will lead to cell lysis, which subsequently leads to diffusion of GSs and the myrosinase through the lysed tissue. Part of them will leach into the cooking water. Enzymatic breakdown of GSs can occur in the lysed tissue as well as in the cooking water as myrosinase can get in contact with GSs. This is usually expected to be limited since the temperature increases quickly and will inactivate myrosinase rapidly, depending to some extent on the stability of the specific myrosinase that is present in the vegetable. Simultaneously, inactivation of myrosinase and thermal degradation of GSs can occur due to the heat.

These preparation methods were reported to reduce considerable amount of GSs in, for example, broccoli (Table 2), white cauliflower [43], Brussels sprouts, and kale [50, 51]. Typically, leaching is the major factor of the loss of GSs during boiling followed by GS thermal breakdown. Canning will have a great impact on the loss of GSs in the products due to the more severe heat treatment. When boiling is performed at higher pressure than the normal one, a higher degree of GS loss was reported [41], although one study reported no significant difference in turnips greens [52].

Although leaching is the major factor of the loss of GSs during boiling the vegetables, the main part of leached GSs can be recovered in the cooking water. In preparation methods that use this water for consumption (e.g., soups), this leaching is not a loss. The rest of the GS loss is likely due to thermal degradation and enzymatic hydrolysis [37, 53]. Contrary to these findings, some other studies suspected that the mechanism of GS thermal breakdown is more dominant than leaching in reducing the GS content [54, 55].

A high retention of GS after boiling was reported in broccoli and Brussels sprouts [43, 56], most likely due to the large size of the vegetable parts and the short boiling time employed. Short heat treatment can result in less-intensive GS loss due to enzymatic hydrolysis, thermal breakdown, and leaching. Most of the hydrolytic enzyme myrosinase will be inactivated and there might be an increase of GS extractability during analysis. D'Antuono et al. [57] reported that the total extracted GS content was twofold higher in boiled cauliflower compared to raw.

Ranges of GS loss after blanching were also reported in broccoli (Table 2) and other *Brassica* vegetables [50, 53, 58, 59]. Higher loss of GSs than boiling can be expected [60] especially when the ratio of water to vegetable is higher, which will lead to more extensive leaching. Moreover, the differences in type of *Brassica* vegetable and blanching technique could influence the behavior of GSs during blanching. Goodrich et al. [58] 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, but these techniques reduced total GS contents in broccoli significantly.

Table 2 Effect of boiling, steaming, and blanching on GS retention in broccoli

		GS retention (%)				
Temperature (C)	Time (min)	Aliphatic	Indole	Aromatic	Total	References
Boiling						
Boiling water	30	17.0	n.a.	50.0	19.4	[38]
	8	126.9	70.5	n.a.	95.3	[43]
	3	126.6	76.1	44.0	90.5	[56]
	5	54.2	51.7	50.0	53.5	[61]
	2; 5	70.3–85.5	71.3–97.5	n.a.	70.4–88.1	[64]
	5	58.8	40.7	n.a.	53.5	[65]
n.a.	10–15	41.5	58.1	Traces	44.2	[50]
	5	56.8	18.4	Up	25.5	[41]
Steaming						
Boiling water	15	124.3	147.0	n.a.	137.0	[43]
	3.5	111.4	104.5	Up	107.1	[41]
	2; 5	93.9–106.9	94.6–119.3	n.a.	96.8–109.6	[64]
	5	93.3	63.2	n.a.	84.5	[65]
100 (oven)	13	133.7	143.0	n.a.	138.9	[43]
Oven	n.a.	89.5	131.9	n.a.	130.8	[62]
~20–~100	2–30	91.5–136.5	44.8–135.1	n.a.	78.9–131.8	[17]
Blanching						
80	3	64.7	97.2	Traces	69.9	[50]
99	4	22.4	11.5	n.a.	17.0	[58]
Steam: 99–102	5.5	72.2	47.2	n.a.	60.1	

n.a. not available

Thus, preparation time and temperature, the ratio of vegetable to water, the preparation method, and the type and geometrical shape of the vegetable tissues are the factors strongly affecting the behavior of GS content during boiling and water blanching.

For steaming, low magnitude of GS loss is expected, since there is no direct contact between the vegetable tissue and the boiling water. The rate of cell lysis, diffusion, leaching, enzymatic breakdown, and thermal degradation are lower than the ones during boiling. Previous studies reported no significant effect of steaming on total GLS content in cabbage and broccoli, cauliflower, and Brussels sprouts [16, 26, 38, 41]. Steaming can increase the accessibility of GSs in cauliflower and broccoli [57, 61, 62]. Nugrahedi et al. [63] reported that duration of steaming affects the behavior of GS content. Total GSs in white cabbage was found to increase during steaming for 10 min followed by a decline during long-term steaming for 180 min. This can be explained by leaching of the GSs to the condensated water layer on the vegetables that is constantly refreshed by condensation and dripping.

4.2.2 Frying

There are two main methods of frying of food, namely, shallow (stir) and deep-fat frying. Stir-frying is commonly performed to prepare *Brassica* vegetable. By using a small amount of hot oil, the vegetable is stir-fried at high temperature for several minutes. This is a very common preparation method to prepare vegetables in Asian countries, usually performed by using a pressure burner to produce a powerful flame used for cooking. Small amounts of water might be added during stir-frying, depending on the local custom, type of *Brassica* vegetable, and the expected product.

Heat from the hot surface of the frying pan is transferred through a thin layer of hot oil to the vegetable. The surface temperature of the vegetable rises rapidly and a proportion of water is vaporized [66]. Compared to other thermal preparation methods, stir-frying applies high temperature of oil and shorter preparation time. Therefore, most of myrosinase is expected to be inactivated. However, the temperature of the main part the vegetable tissue will not exceed 100 °C for the short frying time usually applied since the tissue will still contain most of its water. Overall, low degrees of cell lysis and diffusion, leaching, thermal degradation of GSs, and myrosinase hydrolysis can be expected during short-time stir-frying. On the contrary, deep frying to lower water contents will reduce considerable amount of GSs due to thermal breakdown at the higher product temperatures.

Stir-frying was found to retain the GS content in green cabbage, broccoli, Brussels sprouts, and cauliflower [38, 56] and even increased the extractability of GS in Chinese cabbage [67]. However, Yuan et al. [65] observed stir-frying of broccoli reduced considerable amount of the aliphatic and indole GSs by about 55% and 67%, respectively. Possibly this is due to extensive time-temperature employed. When an amount of water is added during stir-frying, leaching does not significantly contribute to degrade GS content in broccoli as compared to the one without additional water. The authors suspected that thermal breakdown of GSs due to high temperature of stir-frying affects more than leaching [65]. In another study, the effect of various times and temperatures on the GS changes during stir-frying of Chinese cabbage and pak choy was not clearly observed [67].

4.2.3 Microwave Processing

Brassica vegetables contain dissolved ionic contents and considerable amount of water. During the absorption of microwave (MW) energy, the vegetable is heated by rotation of the dipolar water molecules and translation of the ionic components. Heat generated within is transferred throughout the tissue by conduction [68]. The mechanisms affecting the fate of GS content mainly are cell lysis and diffusion, inactivation of myrosinase, and to some extent thermal degradation of GSs.

Magnitude of GS changes is affected by processing time and applied MW power. The longer the processing time, the more the plant cell lysis and thermal degradation will occur. At moderate temperature, myrosinase activity will increase and inactivation will occur rapidly at higher temperature [45]. When considerable amount of water is added to the vegetable, leaching during MW processing can be expected.

Table 3 shows that MW processing affects to various degrees of GS retention in broccoli, depending on the output power and MW time. Fuller et al. [69] and Song and Thornalley [38] reported that MW processing can retain GS content in cabbage, Brussels sprouts, and cauliflower. Verkerk and Dekker [45] found that the combination of output powers, i.e., 180, 540, and 900 W, and various processing times, i.e., over 24 min, lead to little loss of GSs in red cabbage, while some treatments increased the extractable GS content. Therefore, higher accessibility of GS of the plant tissue can be expected during MW heating.

Loss of GSs was reported when water was added for MW processing [41, 64, 70]. Vallejo et al. [41] reported that MW processing for 5 min at 1000 W caused a loss of total GS content of broccoli florets by about 74%, but the recovery of total GSs was only about 1% in water. Although the amount of evaporated water was not reported, GS thermal degradation may play significant role during MW. When a considerable amount of water is lost from the vegetable tissue, the temperature by the MWs can easily increase to values substantially above 100 °C inducing rapid thermal breakdown. A similar microwave processing condition on broccoli, however, reduced only about 18% of total GS content and parts of this loss were recovered in the cooking water [70]. 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 a high power of 1000 W was employed [65].

4.3 Other Processing Methods

4.3.1 Chilling and Freezing

Brassica vegetables can be minimally prepared by cutting or chopping followed by packaging and storing at chilling temperature at 0–5 °C. The products are ready to cook or can be prepared as salads. The combination of low temperature and modified atmosphere packaging can reduce the rate of biochemical and microbiological changes, hence extending the shelf life.

Changes of GSs can be expected due to mechanisms of cell lysis and diffusion, which will lead to enzymatic hydrolysis reaction between GSs and myrosinase. The amount of cell damage due to cutting will depend on the size of the vegetable parts, but is expected to be a low fraction of the total amount of cells in the tissue. Refrigerated storage is expected to inhibit the rate of reaction. Storage conditions at low temperature (<4 °C) and high relative humidity can maintain cellular integrity and preventing the contact of myrosinase and GSs and hence, can retard the loss of GSs [36].

Meanwhile, freezing applies temperatures below the freezing point of the cellular moisture. A proportion of water in the vegetable undergoes a change in state to form ice crystals that can penetrate the cell membranes and cell walls thereby lysing the cells. During frozen storage, this will not cause big changes, but upon thawing rapid GS hydrolysis by myrosinase can occur if the enzyme was not inactivated by blanching prior to the freezing. The extension of shelf life is acquired by a

Table 3 Effect of microwave processing on GS retention in broccoli

			GS retention (%)				
Power (W)	Time (min)	*Brassica* weight (g)	Aliphatic	Indole	Aromatic	Total	References
300	30	10 specimens (floret + stem 2.5 cm)	97.7	110.4	n.a.	102.2	[43]
500; 700; 1000	2.5; 5	150	77.4–110.5	57.6–93.8	62.4–87.1	64.3–97.7	[70]
1100	2; 5	150	83.6–100.0	86.5–118.7	n.a.	83.9–104.1	[64]
1000	5	150	15.9	27.4	Traces	25.5	[41]
1000	5	200	39.7	46.9	n.a.	41.8	[65]
900	0.5–3	20–30	n.a.	n.a.	n.a.	Not sig. loss	[38]

combination of low temperatures, reduced water activity, and pretreatment by blanching whenever applied. Compared to chilling, freezing can retain more GS content of *Brassica* vegetables.

In general, GS content can be best maintained by freezing, provided that myrosinase was inactivated prior to freezing [36]. Freezing the blanched broccoli at −18 °C within 20 min did not substantially change the total GS content but reduced myrosinase activity by 93%. Subsequently, during storage at −20 °C for 90 days, the GS content was generally unaltered [56]. Accordingly, Volden et al. [71] found no significant effects of frozen storage of cauliflower at −24 °C for 12 months on the total GS content. Another study [50] reported that prolonged freezing of blanched *Brassica* vegetables at −22 °C for 48 h did not produce any consistent changes in total GS content. Losses of total GS contents relative to the blanched vegetables were 50.7% and 4.5% in frozen Brussels sprouts and curly kale, respectively. In contrast, total extractable GS contents in frozen green cauliflower and broccoli increased by 20.9% and 28.5%, respectively [50].

The matrix structure of the vegetable tissue can also affect the magnitude of GS changes. Loose structure of the broccoli stalk and flower head are very susceptible to the leaching effects during prior blanching; hence, 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 [72].

Freezing can rupture plant cells and soften vegetables because of water crystallization in extracellular and intracellular spaces within the vegetable matrix. Freeze-thawing fracture of plant cells can disrupt the vegetable tissue to cause extensive cell lysis and diffusion. Subsequently, this will give accessibility of myrosinase to hydrolyze GSs during thawing and eventually cause significant loss of GSs. Song and Thornalley [38] reported that storage of broccoli, Brussels sprouts, cauliflower, and green cabbage at −85 °C for 2 months without prior blanching caused significant loss of GSs upon thawing.

Blanch-freezing as a treatment prior to boiling can enhance the extension of cell lysis and diffusion. This will lead to a high degree of GS loss after boiling [43]. However, Rungapamestry et al. [56] reported no significant change of aliphatic and aromatic GS contents after stir-frying of broccoli when prior blanch-freezing was applied.

4.3.2 Drying

Air drying commonly applies circulation of hot dried air on the surface of the food causing removal of some amount of moisture. High temperature during drying is expected to soften the plant tissue and induce cell lysis and diffusion of components. GS loss will be influenced by enzymatic hydrolysis and thermal degradation [73, 74].

Mrkic et al. [75] reported that different combinations of temperatures at 50 °C through 100 °C and velocities of drying air at 1.2 through 2.25 ms^{-1} affect individual GS in broccoli differently. Compared to the blanched treated only, the remaining GSs after drying are 32–90% for 4-hydroxy-glucobrassicin, 65–92% for glucobrassicin, 29–90% for 4-methoxy-glucobrassicin, and 36–92% for

neoglucobrassicin. Meanwhile, Jin et al. [76] reported that mild air drying of broccoli with the constant air temperatures at 40 °C and 50 °C can retain the glucoraphanin content.

4.3.3 Fermentation

Fermentation is depending on the growth and metabolic activity of lactic acid bacteria, either spontaneously or starter-induced. Salt is usually added to inhibit the growth of undesired microorganisms during the production of fermented products [77]. Sauerkraut is a popular fermented product of *Brassica* vegetable. Some examples of other locally produced fermented *Brassicas* especially from Asia are *Brassica kimchi* from Korea, *dakguadong* from Thailand, *nozawana*-zuke from Japan, *suan-tsai* from Taiwan, and *sayur asin* from Indonesia [21, 78–80].

Compared to the preparation methods on *Brassica* vegetables previously described, the underlying mechanisms affecting GS changes are different. Depending on the production method of fermentation, cell lysis and diffusion, enzymatic hydrolysis, and leaching can occur, which will lead to GS changes. Moreover, changes of GS content in *Brassica* during fermentation can be affected by the type and activity of bacteria, concentration of salt, pH, and fermentation substrate and temperature. Tolonen et al. [77] and Suzuki et al. [21] reported that bacteria and sodium chloride influence on the changes of GS in *Brassica* vegetables during fermentation. Moreover, a concentration of 500 mM NaCl and pH at below 5.5 inactivated myrosinase when analyzed in vitro [21].

In general, fermentation reduces the GS content significantly. In sauerkraut production and storage, there was no GS detected in the product, irrespective of cultivation season on the cabbage, type of fermentation, and concentration of salt [81–83]. The breakdown products of GS were detected, such as ITCs, cyanides, indole-3-carbinol, indole-3-acetonitrile, and ascorbigen [77, 81–83]. It is suspected that the content of the degradation products is not only influenced by the content of the native GS in raw cabbage, but also by to physicochemical properties, such as volatility, stability, and reactivity in an acidic environment, and microbiological stability [81]. Nevertheless, further studies are needed to explain the underlying mechanisms of GS changes during fermentation.

Sarvan et al. [84] and Nugrahedi et al. [85] showed that inactivation of myrosinase prior to the fermentation resulted in an increased retention of GSs in the final product. Heat treatment (i.e., blanching) was applied to the cabbage followed by fermentation (at 25 °C, 4% brine) by *Lactobacillus paracasei* LMG P22043. This treatment retained 35% (27.2 ± 2.3 μmol 100 g^{-1}) of total GSs after fermentation for 71 h as compared to the one before fermentation. Moreover, during refrigerated vacuum storage for 30 days, 23.7 ± 1.5 μmol 100 g^{-1} of GSs still retained [84]. In another fermentation study, raw Indian mustard was withered by microwave processing at 900 W for 2 min to fully inactivate myrosinase. The concentration of sinigrin, the most dominant GS in Indian mustard, can be retained at 30% of the one in the withered leaves, while common fermentation led to considerable loss of GSs. After 7 days of fermentation, about 13% of sinigrin still can be retained [85]

4.3.4 High Pressure Processing

High pressure processing can prolong the shelf life of food by destroying microorganisms. High pressures cause collapse of intracellular vacuoles and damage to cell walls and cytoplasmic membranes [66]. Besides killing microorganism, combination of high hydrostatic pressure and mild temperatures can be an alternative to thermal processing to retain the health-promoting compounds. Thus, treatment on *Brassica* vegetables by a high pressure and temperature combination gives an advantage over other conventional preparation methods [86].

Changes of GS during HPP can be expected due to mechanisms of cell lysis and diffusion, enzymatic breakdown of GS, and possibly also leaching. Nevertheless, the accessibility of GS from the matrices can be improved by this method.

Van Eylen et al. [86] reported that mild pressure processing of broccoli can induce the GS hydrolysis. Moreover, loss of 20% of GS was observed after 35 min of elevated pressure, at 200–300 MPa, and at 20 °C combined treatments. When temperature was increased at 40 °C and the range of pressure was 100–500 MPa, the GS degradation was observed after 15 min, and the greatest GS loss at 63% was obtained at 300 MPa. Thus, the parameters of the process, namely, time, temperature, and pressure, can be varied in order to obtained different amounts of health beneficial products.

5 Product and Process Design

To obtain the highest GS content possible in the processed product as well as to increase the bio-accessibility and bioavailability, the optimization of food preparation and processing methods is inevitably important. At primary production, efforts on cultivation and plant breeding have been performed to increase the level of GSs in fresh *Brassica* vegetables, such as "BroccoCress®" and "Beneforte®." However, since both culinary preparation and industrial processing considerably influence the fate of GSs in the product, there is a need to (re)design and (re)formulate the processing and preparation conditions.

To increase the GS intake in the product, as previously described in Sect. 4.3.3, Sarvan et al. [84] have been redesigned the fermentation method of sauerkraut by inactivation of myrosinase prior to fermentation. Similarly, myrosinase was inactivated prior to fermentation of Indian mustard (*Brassica juncea*) to produce *sayur asin*, a local fermented *Brassica* commonly produced in Asia. [85]. These studies indicate that enzymatic hydrolysis plays an important mechanism underlying the loss of GSs during fermentation. The reformulation strategy was employed during pasta-like production by adding broccoli powder. It was reported that the nutritional function, in terms of GS content, in the pasta and noodle can be improved by enrichment up to 20% (v/v) broccoli powder, and no negative effects on acceptability were observed [87].

To increase the bioavailability, Oliviero et al. [88] have been designed a mild air drying technique to obtain powdered broccoli containing high GSs as well as retaining active myrosinase. By optimizing temperature trajectories, broccoli

product with fully retained GSs and partially retained myrosinase can be obtained. In Sect. 4.3.4, it was shown also that HPP [86] can be considered as a promising technique to increase the bioavailability of GS breakdown products. Moreover, in order to optimize processing and preparation methods, mathematical modeling of the simultaneously occurring mechanisms that influence the fate of GSs can be a valuable tool [25].

6 Conclusions

Processing and preparation have a significant impact on the content and accessibility of GSs in *Brassica* vegetables. Different preparation methods lead to various degrees of GS changes. In general, boiling and fermentation considerably reduce the amount of GSs, while short-term steaming, microwave processing, and stir-frying can retain GS content. Moreover, these preparation methods can increase the accessibility of the compounds from the plant matrix.

A mechanistic approach is valuable to explain and describe the behavior of GSs in *Brassica* vegetables during preparation. By employing this mechanistic approach underlying the GS changes during processing alternative procedures or conditions can be redesigned to improve the retention of GSs. Moreover, reformulation can also be performed to modify the product properties in such a way that the intake of health-promoting GSs increases. Redesigning process and reformulating product can contribute to the aim of improving the diet, especially by employing mathematical modeling techniques.

In addition, by understanding the behavior of GS in Brassica vegetables during processing, a more accurate estimation of the dietary intake of GSs in prepared dishes can be performed. This estimation is important for establishing the relation between intake of phytochemicals and health effects like reducing the risk of certain diseases. However, since GSs do not have health protection effect but the breakdown products, it will be useful to further investigate the breakdown products of GSs in *Brassica* vegetables prior to consumption and the bioavailability of these compounds.

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Dekan Fakultas Teknologi Pertanian Universitas Katolik Soegijapranata, Semarang dengan ini memberikan tugas kepada:

Nama	:	Dr. R. Probo Y. Nugrahedi. M.Sc
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Semarang, 7 Agustu 2016
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