Variation of glucoraphanin and glucobrassicin: anticancer components in Brassica during processing
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Abstract
Effects of cold storage and three common cooking practices, blanching, sautéing, and microwave cooking at different time intervals, on the content of glucosinolate (GSL) anticancer components in six Brassica vegetables were investigated. Eleven GSLs including progoitrin, glucoraphanin, sinigrin, glucoalyssin, gluconapin, glucobrassicanapin, glucoerucin, glucobrassicin, 4-methoxyglucobrassicin, gluconasturtiin, and neoglucobrassicin were quantified using LC-MS and HPLC. Storage at 4 °C indicated no significant loss of GSLs in broccoli, kohlrabi, and cabbage, and approximately 90-100% of the total concentration of aliphatic and indolyl GSLs were detected. Interestingly, glucoraphanin and glucobrassicin, known as a cancer prevention agents, increased approximately above 50% in broccoli, kohlrabi, and cabbage, while the amount of glucobrassicin decreased by 5% in cauliflower for 5 days at 4 °C. Blanching of broccoli at 120 sec significantly (36%) decreased total GSLs; however, sautéing and microwaving decreased by 13-26%. Individual GSLs have different response at blanching. These findings suggest that different processing methods for each vegetable would be preferred to preserve the nutritional qualities.

Keywords: Brassica vegetables; glucosinolates; storage; cooking.

1 Introduction
Brassica vegetables have widely been grown in Europe, Korea, Japan, and China for at least 600 years and in North America since the early 20th century (HORN, 1985). Consumption of Brassica vegetables as food has been increased because of their relatively high protein content and high dry matter digestibility and also because of their role in prevention of cancer (LI et al., 2010). Among Brassica vegetables, broccoli (Brassica oleracea var. italica), cabbage (B. oleracea var. capitata), cauliflower (B. oleracea var. botrytis), and kale (B. oleracea var. acephala), stand out as good sources of phytochemicals such as glucosinolates (GSLs), vitamins, carotenoids, and polyphenols. These vegetables are differentiated from other plants by their high GSL contents (CARTEA; VELASCO, 2008). GSLs, known as mustard oil glucosides, are a class of nitrogen and sulphur-containing natural products distributed in 16 dicotyledonous families of the order Capparales and are mostly clustered within the Brassicaceae (VERKERK et al., 2009). The anti-carcinogenic properties of Brassica vegetables have mainly been attributed to the hydrolytic products rather than to the intact GSLs (Figure 1). Most hydrolysis products from glucoiberin, sinigrin, and progoitrin have anticancer effects. This chemo-preventive activity of Brassica vegetables is related to the amount of GSLs, their conversion to isothiocyanates, bioavailability, and stability of the isothiocyanate metabolites.

Brassica vegetables are normally purchased from local stores or obtained from farms and stored at 4-8 °C in a refrigerator or at ambient temperature for up to a week. Storage and cooking process of Brassica vegetables commonly consumed daily by humans affect the GSL arrangement and related isothiocyanate content (JIA et al., 2009). GSLs are chemically stable until they come in contact with the enzyme myrosinase, which is stored compartmentalized, separated from GSLs in the plant tissue (KELLY; BONES; ROSSITER, 1998). They become accessible to myrosinase when the plant tissue is disrupted, such as in insect predation, or when vegetables are frozen and thawed, chopped, and shredded in preparation for cooking (VERKERK; DEKKER; JONGEN, 2001). Domestic treatments including chopping, cooking, steaming, and microwaving have wide impact on GSL content, while the effects of industrial processes such as freezing, fermenting, and hot packing have little effect (DEKKER; VERKERK; JONGEN, 2000). During heating, GSL levels are reduced by enzymatic breakdown, thermal breakdown, and leak into the hot medium. Thermal breakdown of synthetic glucobrassicin results in 10% degradation and leads to the formation of a new breakdown product, 2-(30-indolylmethyl) glucobrassicin, after heating at 100 °C for 1 hour (CHEVOLLEAU et al., 2002). Microwaving is an efficient choice for cooking vegetables due to the low amount of cooking
2.1 Chemicals

HPLC-grade acetonitrile (CH₃CN) and methanol (MeOH) were purchased from J. T. Baker Chemical Co. (Phillipsburg, NJ, USA) and sodium acetate (NaC₂H₃O₂·3H₂O) from Hayashi Pure Chemical Industries, Ltd. (Osaka, Japan). DEAE-Sephadex A-25 and Aryl sulfatase (type H-1, EC 3.1.6.1) were obtained from Sigma-Aldrich (St Louis, MO, USA). Standard sinigrin (2-propenyl GSL) (purity 99%) was purchased from Sigma-Aldrich (St Louis, MO, USA).

2.2 Plant materials

Freshly harvested broccoli, cauliflower, white and red kohlrabi, and white and red cabbage originally cultivated in the Jeju Island, Republic of Korea, were purchased from a local market, the Agricultural and Fish Market. They were free from insect and mechanical damage. The vegetables were transported to the laboratory within 30 minutes of purchasing and stored at 4 °C until chemical analysis. Fresh broccoli and cauliflower were cleaned by removing of inedible parts and then chopped into homogeneous pieces leaving a stem of about 5 cm. The outer leaves of cabbage and kohlrabi were trimmed. To obtain more homogeneous samples, each batch was then properly divided into > 10 equal portions. One portion was retained raw, and the others were prepared using different cooking methods, in triplicate, as given below.

2.3 Effect of storage

Effect of storage on the GSL contents was evaluated by storing vegetables up to 10 days at 4 °C in the vegetable compartment of a domestic refrigerator (Samsung Electronics Co. Ltd., Korea). Individual GSL contents were analyzed at 0, 1, 3, and 5 days for broccoli and cauliflower, respectively, and at 0, 1, 3, 6, and 10 days for kohlrabi and cabbage.

2.4 Heat treatment of vegetables

For blanching, fresh raw vegetables were added individually to boiling tap water in a covered stainless steel pot (1000 cm³) and cooked on a moderate flame for 30, 90, and 120 sec. Cooking time was measured from the time the samples were placed into the boiling water; the vegetables were boiled and subsequently drained off for 30 sec.

For studies of sautéing by stir-frying, a non-stick pan was used (diameter of 25 cm; depth of 5 cm). An aliquot (20% w/w of fresh vegetable) of soybean cooking oil was preheated to 200 °C. Fresh vegetable were sliced into 1 cm strips immediately beforehand, added to the oil, and stir-fried for 90 sec with repeated stirring using a wood stick during cooking. The temperature of the oil decreased to 100-130 °C with the addition of the vegetables, and it was maintained at this temperature during cooking. After cooking, the vegetables were taken out, cooled to room temperature, and analyzed for GSLs.

Microwave cooking treatments were carried out using a domestic microwave oven (Samsung Electronics Co. Ltd., Korea) without water. The microwave oven was operated at a frequency of 2450 Hz at power (800W). Fresh broccoli was cooked for 90 sec.

2.5 Extraction of crude glucosinolates and their desulphation

Crude glucosinolates were extracted according to the Official Procedure ISO 9167 (INTERNATIONAL...., 1992), as described earlier by Kim, Jin, Ishii (2004). Briefly, 100 mg of freeze-dried materials were extracted with 1.5 ml of boiling 70% (v/v) methanol in a water bath at 70 °C for 5 minutes. After centrifugation (12,000 rpm, 4 °C, 10 minutes), the resulting supernatant was collected, and the residue was extracted twice. The combined supernatant was taken as the crude of GSLs. Desulphation of the crude GSL extracts was performed using a DEAE anion exchange column, which was prepared by adding slurry of DEAE Sephadex A-25 previously activated with 0.5 M sodium acetate. Five ml of sinigrin solution (0.1 mg/ml), used as an external standard, was separately desulphated using the same DEAE anion exchange column. The crude GSL extracts were loaded onto a pre-equilibrated DEAE anion exchange column. After washing with 1 ml (× 3 times) of ultrapure water to remove cation and neutral ions, 75 µl of aryl sulfatase (E.C.3.1.6.1) were then loaded onto each column. After desulphation reaction overnight (16-18 hours) at room temperature, the desulphated

Figure 1. Glucosinolates and their degradation products.
GSLs were eluted with 0.5 ml (×3 times) of distilled water. The eluates were filtered through 0.45 μm Teflon PTFE syringe filter and analyzed immediately by HPLC or stored at 4 °C in the refrigerator until chemical analysis.

### 2.6 Separation and identification of desulpho (DS)-glucosinolates using HPLC and LC-MS

DS-GSLs were analyzed by a 1200 series HPLC system (Agilent Technologies, CA, USA) equipped with an Inertsil ODS-3 (C18) column (150 × 3.0 mm i.d., 3 μm particle size) (GL Science, Tokyo, Japan). The HPLC analysis was carried out with a flow rate of 0.2 ml min⁻¹ at column oven temperature of 40 °C and a wavelength of 227 nm. The solvent system employed was (A) ultrapure water (PURELAB Option-Q, ELGA) and (B) 100% acetonitrile. The solvent program was as follows: 0 minute solvent B 7%, 18 minutes solvent B 24%, then kept constant at solvent B 24% by 32 minutes, further solvent B 7% at 32.1 minutes, and then kept constant at solvent B 7% for 10 minutes (total 40 minutes). The individual GSLs were quantified with sinigrin solution according to their HPLC area and response factor (INTERNATIONAL..., 1992; CLARKE, 2010). In this study, all the DS-GSLs were determined as GSLs.

Electrospray ionization (ESI)- mass spectrometry was used for the identification of individual DS-GSLs with an API 4000 Q TRAP system (Applied Biosystems, Foster City, CA, USA) in positive ion mode ([M+H]⁺) that was equipped with an Agilent 1200 series HPLC system. The MS operating conditions were as follows: scan range, m/z 100-800 (scan time, 1.0 sec); curtain gas (20 psi), nebulizing gas (50 psi), heating gas (50 psi) by high purity nitrogen (N₂); heating gas temperature, 550 °C; ion spray voltage, 5,500 V; declustering potential, 100 V; and entrance potential, 10 V.

### 2.7 Statistical analysis

GSLs were analyzed with three replications. The mean and standard deviation of all data were calculated in Excel 2007.

### 3 Results and discussion

#### 3.1 Identification of individual glucosinolates

In this study, 11 GSLs of all cruciferous vegetables were tentatively identified by LC-MS/MS. The molecular ion and fragmentation patterns of MS spectra were in accordance with the data found in the literature and allowed for unequivocal identification of GSLs (BARBIERI et al., 2008). The identified GSLs were quantified against the external standard (sinigrin) using relative response factors derived from pure GSL standards (INTERNATIONAL..., 1992; CLARKE, 2010). The response factors used are shown in Table 1 with respect to the commercially available sinigrin. The identified GSLs response factors varied 5.8 fold. Glucoraphanin shared the highest values (1.15), and neoglucobrassicin had the lowest value (0.2). In general, modifications of the side chain by elongation, oxidation of the parent thiol function, or alkene formation had little effect on the response factor. Seven aliphatic GSLs (progoitrin, glucoraphanin, sinigrin, glocoalyssin, glucobrassicanapin, and glucoraphanin, and glucobrassicin), three indolyl GSLs (glucobrassicin, 4-methoxyglucobrassicin, and neoglucobrassicin), and one aromatic GSL (glucosinapterin) were identified based on the fragmentation patterns of MS spectrum and quantified based on the retention times of HPLC chromatograms (Table 1, Figure 2). These findings were in agreement with those of other research groups claiming that glucoraphanin, sinigrin, glucobrassicin, and neoglucobrassicin were commonly found in cabbage varieties (CARTEA et al., 2008).

### 3.2 Effect of storage periods

The three most abundant GSLs (glucoraphanin, glucoalyssin, and glucobrassicin) were found in the broccoli on the first day of storage (0) (Table 2). In a previous study, we reported that the predominant GSL in Brassica crops was glucoraphanin, which represents 51% and 77% of total GSL and total aliphatic contents, respectively (KIM et al., 2002). Interestingly, after 5 days of storage, glucoraphanin and glucobrassicin contents in broccoli increased 68 and 31%, respectively.

#### Table 1. Identification of glucosinolates from Brassica vegetables.

| No. | Retention time (min) | Trivial names          | Semisystemic names of R-group | Structures of R-group | Molecular weight (a) | Response factors (b) |
|-----|----------------------|------------------------|--------------------------------|-----------------------|----------------------|---------------------|
| 1   | 5.39                 | Progoitrin             | 2-Hydroxy-3-butenyl           | CH₂(C=CHCH(OH)CH₂)₂⁻ | 309                  | 1.09                |
| 2   | 6.18                 | Glucoraphanin          | 4-Methylsulfinylbutyl         | CH₂(SO-CH₂)₃⁻        | 357                  | 1.07                |
| 3   | 7.75                 | Sinigrin               | 2-Propanyl                    | CH₂=CHCH₂            | 279                  | 1.00                |
| 4   | 8.74                 | Glocoalyssin           | 5-Methylsulfinylpentyl        | CH₂(SO-CH₂)₃⁻        | 371                  | 1.07                |
| 5   | 14.23                | Glucobrassicin         | 3-Butenyl                     | CH₂=CH(C=CH₂)₃⁻      | 293                  | 1.11                |
| 6   | 16.24                | Glucobrassicinapain    | 4-Pentenyl                    | CH₂=CH₂(C=CH₂)₃⁻     | 307                  | 1.15                |
| 7   | 20.78                | Glucoalyssin           | 4-Methylthiobutyl             | CH₂S-(CH₂)₃⁻         | 341                  | 1.04 (d)            |
| 8   | 22.79                | Glucobrassicin         | 3-Indolomethyl                | Indole-3-CH₂⁻        | 368                  | 0.29                |
| 9   | 25.23                | 4-Methoxyglucobrassicin| 4-Methoxy-3-indolomethyl      | Indole-4-OCH₂⁻       | 398                  | 0.25                |
| 10  | 26.22                | Glucoraphanin          | 2-Phenylethyl                 | C₆H₅-C=CH₂⁻          | 343                  | 0.95                |
| 11  | 31.09                | Neoglucobrassicin      | 1-Methoxy-3-indolomethyl      | Indole-1-OCH₂⁻       | 398                  | 0.20                |

(a) No.: The elution order of GSLs of HPLC profiles (Figure 2). (b) As a desulpho-glucosinolate. (c) ISO 9167-1 (INTERNATIONAL..., 1992). (d) Clarke (2010).
Figure 2. HPLC profiles of desulpho-glucosinolates isolated from cruciferous vegetables. (a), broccoli; (b), cauliflower; (c), white kohlrabi; (d), red kohlrabi; (e), white cabbage; (f), red cabbage. Peak numbers refer to the GSLs listed in Table 1. Peak 1, Progoitrin; 2, Glucoraphanin; 3, Sinigrin; 4, Glucoalyssin; 5, Gluconapin; 6, Glucobrassiccanapin; 7, Glucoerucin; 8, Glucobrassicin; 9, 4-Methoxyglucobrassicin; 10, Gluconasturtiin; 11, Neoglucobrassicin.
The storage period affected the content of selected GSL in cauliflower to a different extent. The changes in the amount of main GSL, such as sinigrin, glucoraphanin, and glucobrassicin, irrespective of the time of storage, did not exceed 10%. Individual GSL contents of cauliflower showed a significant decrease in sinigrin and glucoraphanin after 5 days of storage. Similarly, Jeffery et al. (2003) reported that indolyl GSLs were more sensitive to storage conditions than the aliphatic or aromatic GSLs.

The content of GSLs in raw white kohlrabi, red kohlrabi, white cabbage, and red cabbage were determined (Table 3 and 4). The highest total content of the GSLs was found in white cabbage approximately 61.76 µmol/g dry weight (DW) and lowest in white kohlrabi 16.72 µmol/g DW. Glucoraphanin and glucoerucin were the major GSLs in kohlrabi 32.04 and 8.10 µmol/g DW, with lower contents than the other GSLs. After 5-10 days of storage at 4 °C, a sharp increase in GSL content was observed in kohlrabi and cabbage with significant changes 16-18% in total GSL levels (Table 3 and 4). An increase of 100% in the total aliphatic GSLs and 90% in total indolyl GSLs was detected. After storage for 3 days, total GSL content increased 38% in white cabbage. For individual GSLs in white cabbage, the losses of glucoraphanin, sinigrin, and glucobrassicinap in were higher than that of progoitrin, glucoerucin, and glucobrassicin.

When stored at 4 °C, there was no significant decrease in the GSL content of the Brassica vegetables studied. In contrast, the total and individual GSL contents increased proportionally during storage for 10 days. Some of the GSLs showed significant loss after 5 days. Similarly, glucoraphanin and glucoiberin contents increased 25% for broccoli stored at 10 °C for 7 days (HANSEN et al., 1997). Storage of vegetables at 4 °C may cause a significant increase of GSLs due to secondary metabolite synthesis in plant cells sentence. Humidity is known as a critical factor in GSL retention and increment when postharvest temperatures rise or are kept at approximately 4 °C. Based on the results obtained, it can be stated that extended storage of vegetables causes a successive increase in the GSL content, which is more intense within the first three days of storage. Storage at 4 °C indicated no significant loss of GSLs in broccoli, kohlrabi, and cabbage; however, an increase of 100% in the total aliphatic and indolyl GSLs was observed. It is suggested that the storage of broccoli, kohlrabi, and cabbage for a short period of about 10 days did not change the GSL contents, while the storage of cauliflower at 4 °C is will not retain the anticancer valuable

Table 2. Glucosinolate contents (µmol/g dry wt., n=3) in broccoli and cauliflower stored at 4 °C for 5 days in the refrigerator.

| No. | Broccoli | Cauliflower |
|-----|----------|-------------|
|     | 0³       | 1           | 3           | 5           |
| 1   | 0.81±0.24 | 1.22±0.08a | 0.70±0.04a  | 1.71±0.77a  |
| 2   | 19.05±3.18 | 25.99±1.51a | 18.63±1.42a | 31.95±11.56a |
| 3   | 2.50±0.20  | 3.36±0.68a  | 2.59±0.08a  | 3.67±0.80a  |
| 4   | 1.02±0.34  | 1.04±0.25a  | 0.87±0.17a  | 1.19±0.21a  |
| 5   | 3.15±0.58  | 3.57±1.01a  | 3.09±0.41a  | 3.48±1.04a  |
| 6   | 5.80±3.42  | 5.96±3.74a  | 6.78±2.70a  | 6.88±0.93a  |
| 7   | 0.63±0.23  | 0.75±0.28a  | 0.61±0.09   | 0.74±0.22a  |
| 8   | 19.57±3.72 | 21.28±1.77a | 21.72±1.54a | 25.61±5.08a |
| 9   | 5.35±0.08  | 5.68±0.36ab | 5.96±0.45b  | 6.62±0.66a  |
| 10  | ND        | ND          | ND          | ND          |
| 11  | 3.23±0.58  | 2.93±0.09b  | 4.41±0.58b  | 6.71±1.06a  |
|     | Total     | 61.11±4.14b | 71.79±6.82ab | 65.35±3.17b |

³The trivial names of glucosinolates are shown in Table 1. ³ Duration of storing period (days). ³ ND, not detected.

Table 3. Glucosinolate contents (µmol/g dry wt., n=3) in white and red kohlrabi stored at 4 °C for 10 days in the refrigerator.

| No. | White kohlrabi | Red kohlrabi |
|-----|----------------|-------------|
|     | 0³  | 1 | 3 | 6 | 10 | 0³  | 1 | 3 | 6 | 10 |
| 1   | 1.13±0.30a | 1.14±0.81a | 0.97±0.38a | 1.93±1.54a | 2.80±1.77a | 1.73±0.93a | 2.30±1.35a | 2.39±1.42a | 1.99±1.07a | 1.18±0.73a |
| 2   | 2.11±0.65a | 2.32±1.33a | 2.40±0.24a | 3.21±0.93a | 4.39±2.36a | 2.14±1.59a | 2.55±2.21a | 2.55±2.13a | 3.47±1.53a | 1.61±0.57a |
| 3   | 2.95±1.16a | 3.06±2.18a | 2.66±0.10a | 5.01±2.88a | 5.50±4.13a | ND   | ND   | ND   | ND   | ND   |
| 4   | ND³ | ND | ND | ND | ND | ND | ND | ND | ND | ND |
| 5   | ND | ND | ND | ND | ND | ND | ND | ND | ND | ND |
| 6   | 5.32±1.67a | 8.68±6.36a | 13.10±6.68a | 10.80±1.17a | 23.00±14.24a | 2.04±1.23a | 3.66±8.70a | 3.31±11.72a | 25.85±8.47a | 28.42±9.23a |
| 7   | 2.97±1.13a | 4.21±1.51a | 4.39±2.06a | 5.30±3.71a | 6.86±4.26a | 7.47±5.59 | 10.11±9.08a | 8.59±6.72a | 11.86±6.28a | 9.90±6.95a |
| 8   | 1.34±0.64a | 1.67±0.81a | 2.48±1.68a | 2.35±0.93a | 4.15±1.74a | 8.10±3.87a | 9.38±4.83a | 8.22±5.58a | 8.35±2.17a | 6.28±3.00a |
| 9   | 0.56±0.20a | 0.61±0.27a | 0.78±0.44a | 0.81±0.16a | 1.07±0.04a | 0.82±0.55a | 0.72±0.39a | 0.88±0.53a | 1.03±0.64a | 0.70±0.40a |
| 10  | ND | ND | ND | ND | ND | ND | ND | ND | ND | ND |
| 11  | 0.35±0.13a | 0.55±0.35a | 0.56±0.36a | 0.70±0.36a | 0.57±0.09a | 0.86±0.21a | 0.99±0.22a | 1.54±0.88a | 1.43±0.63a | 1.18±0.58a |
| Total | 16.72±3.39a | 21.85±13.26a | 27.36±9.50a | 30.12±15.80a | 48.35±27.29a | 51.71±14.77a | 63.77±16.05a | 59.16±16.67a | 54.92±21.17a | 50.03±16.49a |

³The trivial names of glucosinolates are shown in Table 1. ³ Duration of storing period (days). ³ ND, not detected.
nutritional compounds such as glucoraphanin. It is necessary to evaluate and quantify aliphatic GSLs (glucoraphanin, gluconapin, and glucosein) and indolyl GSL (glucobrassicin) because of their anticancer activity.

3.3 Effect of cooking on glucosinolate contents

Among the Brassica vegetables studied, broccoli and cauliflower are the best sources of anticancer agents. Cooking has been widely reported to cause a decrease in GSLs in broccoli and cauliflower, but varied results have been found (JONES; FARAGHER; WINKLER, 2006). Blanching affected the content of GSLs in broccoli and cauliflower to a different extent. Total GSL contents in the broccoli blanched for 120 sec were significantly (36%) decreased, whereas, in cauliflower even after 120 sec, total GSL did not change (Table 5). The levels of indolyl GSLs, glucobrassicin, and neoglucobrassicin, reduced by approximately 31-65% in both broccoli and cauliflower. The changes in the contents of main indolyl GSL glucobrassicin, except for broccoli blanched for 90-120 sec, were comparable. In the group of aliphatic GSLs, the biggest changes during the first 60 sec of blanching were observed for glucoalyssin (about 100%), and the smallest was observed for sinigrin (> 10%) in cauliflower. However, the rate of the content loss of glaconasturtiin was lower than that of sinigrin, which indicates higher thermostability of sinigrin. Blanching of broccoli and cauliflower leads to inactivation of myrosinase and decomposition of thermo-labile GSL, especially indolyl GSLs. In addition, diffusion of water results in major losses of GSL during boiling. The reduction in the amount of glucobrassicin and glucobrassicinapin in broccoli was similar; however, during blanching their contents were the highest, ranging from 30-33%. The contents of glucobrassicin and glucoraphanin in cauliflower were lower ranging from 4-15% of their contents in raw cauliflower. An increase in the content of glucoraphanin after 120 sec of blanching with similar amounts of progoitrin may suggest that GSL can be bound to cell walls and released after deep disintegration of cell structures. Between 60 and 90 sec, the content of glucobrassicin and sinigrin increased 37% and 25%, respectively. The amount of glucobrassicin and glucoraphanin increased about 10%. During the last 30 sec of blanching, the content of glucobrassicin decreased about 3% and glucoraphanin decreased 17%. Jones, Faragher and Winkler (2006) also reported that inactivation of myrosinase in cruciferous vegetables, as an effect of boiling, may limit GSL

Table 4. Glucosinolate contents (µmol/g dry wt., n=3) in white and red cabbage stored at 4 °C for 10 days in the refrigerator.

| No. | Blanching | Saturing | Microwave cooking |
|-----|-----------|----------|-------------------|
|     | 0         | 1        | 2                 |
| 1   | 5.68±2.50a | 7.01±4.48a | 7.04±3.01a        |
| 2   | 4.24±0.96a | 5.66±3.39a | 6.62±3.39a        |
| 3   | 10.55±4.34a| 14.17±5.52a| 13.00±3.97a       |
| 4   | ND        | ND       | ND                |
| 5   | 1.01±0.44a | 1.17±0.31a | 1.07±0.33a        |
| 6   | 1.77±0.79a | 2.67±0.74a | 2.67±0.74a        |
| 7   | 0.69±0.21a | 1.19±0.48a | 1.19±0.48a        |
| 8   | 21.45±7.27a| 24.06±5.66a| 24.06±5.66a       |
| 9   | 6.85±1.38a | 7.68±1.03a | 7.68±1.03a        |
| 10  | 0.76±0.39a | 1.07±0.25a | 1.07±0.25a        |
| 11  | 0.18±0.06a | 0.21±0.08a | 0.22±0.02a        |

Total 53.17±12.70a 57.55±9.08a 61.76±12.01a

Table 5. Glucosinolate contents (µmol/g dry wt., n=3) of broccoli from difference of recipe and cauliflower by blanching time.

| No. | Blanching | Saturing | Microwave cooking |
|-----|-----------|----------|-------------------|
|     | 0         | 1        | 2                 |
| 1   | 9.95±3.18a | 16.72±1.83a| 16.72±1.83a       |
| 2   | 2.67±0.74a | 2.67±0.74a | 2.67±0.74a        |
| 3   | 0.69±0.38a | 0.76±0.28a | 0.76±0.28a        |
| 4   | 21.45±7.27a| 21.71±1.34a| 21.71±1.34a       |
| 5   | 6.85±1.38a | 7.68±1.03a | 7.68±1.03a        |
| 6   | 0.76±0.39a | 1.07±0.25a | 1.07±0.25a        |
| 7   | 0.18±0.06a | 0.21±0.08a | 0.22±0.02a        |

Total 53.17±12.70a 57.55±9.08a 61.76±12.01a

*The trivial names of glucosinolates are shown in Table 1.* Duration of storing period (days). ND, not detected.
decomposition, thus resulting in consumption of GSL instead of their breakdown products by humans. In this study, most of the GSL structures present in cauliflower were tightly packed on the cell walls and were released after significant disintegration caused by the extended cooking period.

Sautéing and microwave cooking significantly reduced (13-26%) total GSL contents in broccoli, regardless of the cooking time. With regard to microwaving, there was a significant change in the total GSLs, but only slight changes in single GSL content. Similarly to the findings of Miglio et al. (2008), the total content of GSLs of fresh broccoli significantly decreased in both cooking methods, whereas it slightly changed during microwaving since the main compound (glucoraphanin) slightly increased (14%) and indolyl GSL significantly decreased (28%) in both cooking methods. This change in GSLs is probably justified by the lack of water in the microwaving and sautéing cooking methods, confirming that the great loss of these compounds is due to high cooking water evaporation containing leached compounds. However, the changes in the GSL content upon microwave cooking was significantly different between aliphatic and indolyl compounds. In the initial processes, myrosinase activates the degradation of GSLs to release different breakdown products. Generally, indolyl GSLs form metabolites that are inhibitors of carcinogenesis, whereas aliphatic GSL decomposes into volatile isothiocyanates, which are responsible for pungent or bitter taste. The levels of GSL do not necessarily decrease rapidly as a result of vegetable chopping and cooking and even induction can take place. The levels of indolyl GSLs and some aliphatic GSLs increased after chopping of Cruciferae vegetables. The biological activities that take place due to vegetable processing are: hydrolysis of GSLs by a hydrolytic enzyme myrosinase; loss of enzymatic cofactors such as ethiopsiferic protein; thermal breakdown and/or leaching of GSLs and their metabolites or volatilization of metabolites; induction of indolyl GSLs was not confirmed (MITHEN, 2001). A hypothesis assuming that the possibility of the binding of GSL with the cell walls as in the case of other low-molecular compounds, e.g., saccharides, glycosides, and inositol phosphates, would not be inconsistent with a theory assuming that the GSL present in the vacuole is the main reservoir of the cell (LUTHY; MATILE, 1984).

4 Conclusions

This study demonstrated that individual GSLs have different response to different cooking methods and cultivars. Although broccoli and cauliflower belong to the same Brassica family, fresh broccoli tend to lose the GSLs during blanching, sautéing and microwaving, while the GSLs of cauliflower, such as glucoraphanin, increased during blanching for 120 sec. It can be concluded that during the processing of vegetables, aliphatic GSL is generally more stable than indolyl GSL, thus indicating that the relative stabilities of individual GSLs may be a function of their respective chemical structures.

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