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Effects of some technological processes on glucosinolate contents in cruciferous vegetables

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Abstract

Effects of blanching, boiling and freezing of selected cruciferous vegetables (Brussels sprouts, white and green cauliflower, broccoli, and curly cale) on their glucosinolate (GLS) contents were determined. It was found that blanching and cooking of the vegetables led to considerable ($P < 0.05$) losses of total GLS, from 2.7 to 30.0% and from 35.3 to 72.4%, respectively. No systematic changes in total GLS were found in the vegetables that were blanched and frozen for 48 h. In addition, the highest concentration of cancer-protective compounds, such as aliphatic and indole GLS, were found in Brussels sprouts (sinigrin and glucobrassicin) and in broccoli (glucoraphanin).

Keywords: Glucosinolates; Cruciferous vegetables; Blanching; Cooking; Freezing

1. Introduction

Glucosinolates (GLS) are a group of natural non-nutrient compounds with important therapeutic, notably cancer-protective, properties. These compounds are present mainly in plants belonging to the family Brassicaceae (former Cruciferae) (McNaughton & Marks, 2003). The largest and most commonly eaten plants within the family Brassicaceae are the cruciferous vegetables of the Brassica genus. These vegetables are distinguished from other plants by their high GLS contents (Duncan, 1991; McNaughton & Marks, 2003). Also, they differ among each other in their GLS contents and profiles. The above differences result from genetic and environmental (agronomic and climatic conditions) factors. Interestingly, any physical damage to cruciferous plants, may increase the GLS, which act as secondary metabolites that protect these plants against potential pests (Ciska, Martyniak-Przybyszewska, & Kozłowska, 2000; Duncan, 1991; Hrncirik & Velisek, 2000).

Glucosinolates are unstable compounds that are hydrolysed to thiocyanates, isothiocyanates and nitriles, due to the action of myrosinase (thioglucoside glucohydrolase EC 3.2.3.1) present in plant tissue (Hrncirik & Velisek, 2000; McNaughton & Marks, 2003; Zhang, Talalay, Cho, & Posner, 1992). Getahun and Chung (1999) have indicated that, regardless of myrosinase action, GLS can be converted into their biologically active compounds in the alimentary tract. Thus, the effectiveness of GLS consumed by a human being may be influenced by conversion of these compounds into thiocyanates, isothiocyanates and nitriles in the digestion process (Dekker, Verkerk, & Jongen, 2000; Shapiro, Fahey, Wade, Stephenson, & Talalay, 1998).

The above breakdown products of myrosinase (notably thiocyanates, isothiocyanates), by modulating the activity of Phase I and Phase II xenobiotic metabolizing enzymes, may exert cancer-preventive action (Jongen, 1996; Nestle, 1998; Talalay & Fahey, 2001; Van Poppel, Verhoeven,

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Verhagan, & Goldbohm, 1999). Consequently, adequate consumption of cruciferous vegetables has resulted in their anticarcinogenic action. However, there are few reports that suggest opposite effects of myrosinase breakdown products (Verhoeven, Goldbohm, van Poppel, Verhagen, & van den Brandt, 1996). It is also believed that some myrosinase breakdown products, especially progoitrin, converted further to goitrin (5-vinyl-2-oxazolidinethione), may lead to endemic goitre, due to hypothyroidism. Moreover, nitriles may also be harmful to humans by leading to liver and kidney hypertrophy (Duncan, 1991; Schöne, Kirchheim, & Schumann, 1994; Schöne, Rudolph, Kirchheim, & Knapp, 1997).

In spite of the above negative effects, associated with consumption of cruciferous vegetables, McNaughton and Marks (2003) underlined the problem of their insufficient consumption as sources of GLS. In more detail, the consumption of broccoli and cauliflower, rather than white and savoy cabbage, was emphasised (Nugon-Baudon & Robot, 1994).

Technological processes, such as blanching, boiling and freezing, affect GLS content in cruciferous vegetables, their breakdown, and taste and aroma of final products (McNaughton & Marks, 2003; Schöne et al., 1994). In view of the above, the objectives of this paper were to evaluate the content of total and selected individual GLS (notably the major biologically active compounds) in common cruciferous vegetables (Brussels sprouts, white and green cauliflower, broccoli and curly cale). Moreover, the objective of this paper was to evaluate effects of blanching, cooking and freezing on the changes in contents of total and selected GLS, in the processed vegetables.

2. Materials and methods

2.1. Material

Five common cruciferous vegetables: Brussels sprouts (cv. Maczuga), white cauliflower (cv. Rober), green cauliflower (cv. Amfora), broccoli (cv. Sebastian) and curly kale (cv. Winter Bol), were obtained from Krakowska Hodowla i Nasiennictwo Ogrodnicze "Polan", in 2004.

The samples of studied vegetables were taken for analyses directly from the field. The plants of each vegetable (5 kg green mass) were cut vertically into four or eight pieces. This material was divided into four sub-samples, which were subsequently used to obtain (a) fresh, (b) blanched, (c) boiled and (d) frozen (blanched and stored for 48 h at −22 °C) vegetables. The fresh vegetables were obtained by washing in running water, drying on filter paper, crushing and then freezing at −22 °C. After freezing, this sub-sample was immediately freeze-dried using a Christ Alpha 1-4 freeze-dryer, and ground, using a Tecator Knifetec 1095 Sample Mill, until uniform powder was obtained. The blanching was carried out at 80 °C for approximately 3 min to inactivate the enzymes present in this sub-sample and the boiling was carried out for 10–15 min. The blanched and boiled vegetables were then prepared as described for the fresh vegetables. The frozen vegetables (i.e., blanched and stored for 48 h at −22 °C), were freeze-dried and ground as described above.

2.2. Analytical methods

Analyses of glucosinolates (GLS) were done in fresh, blanched, boiled and frozen vegetables. GLS were extracted from the material according to the method reported in the Official Journal of European Communities (1990). Briefly, duplicate 200 mg samples of freeze-dried material were extracted thrice with boiling 70% methanol. Since all cultivars examined lacked glucotropaeolin, a known amount was added to each sample, just before the first extraction, as an internal standard for the HPLC analysis. The isolation, desulphatation and HPLC of GLS were carried out according to the modified method of Heaney, Spinks, Hanley, and Fenwick (1986). Desulpho-GLS were separated on the HPLC system with an auto injector (20 μl loop), Spherisorb ODS-2 3 Micron column (150 × 4.6 mm) and 1.2 ml/min flow rate at 32 °C by eluting with a gradient of water (A) and 20% acetonitrile (B) as follows: isocratically 1% B for 1 min, gradient to 99% B for 30 min (curve 3), isocratically 99% B for 6 min, linear gradient to 1% B for 5 min, 1% B for 8 min. GLS were detected at $\lambda = 229$ nm. The content of GLS was quantified using the internal standard and relevant relative response factors (Official Journal of European Communities, 1990).

2.3. Statistical analysis

The results were expressed as treatment means. The data were subjected to two-factorial (4 × 5) analysis of variance, including four processing methods and five species (cultivars) of cruciferous vegetables. The differences between treatment means were evaluated using Duncan's multiple range test. Treatment effects and differences between treatment means were considered significant at $P < 0.05$. The Statistica 6.1 software package (StatSoft, Tulsa, OK) was used for statistical analysis.

3. Results and discussion

3.1. General

The HPLC analyses of the material identified the presence of 14 various glucosinolates (GLS) in all species and cultivars of cruciferous vegetables under study, including nine aliphatics, four indoles and one aralkyl compound (Table 2). At the same time, total GLS concentrations were affected by both, species of cruciferous vegetables (Brussels sprouts, white and green cauliflower, broccoli and curly cale) and processing methods (blanching, boiling and freezing).

Table 1
Results of two-way analysis of variance for changes in glucosinolate (GLS) contents in cabbage vegetables exposed to various technological processes (mg/100 g)

	Vegetables				$\bar{x}$
	Raw	Blanched	Boiled	Frozen	
Brussels sprouts	128.0 r	92.1 q	47.1 k	45.4 j	63.7
White cauliflower	34.1 f	29.7 d	22.0 a	31.2 e	29.2
Green cauliflower	38.1 h	37.1 g	21.9 a	44.9 j	35.5
Broccoli	60.1 m	41.8 i	26.4 c	53.7 l	45.5
Curly cale	84.5 o	66.6 n	23.4 b	63.6 m	59.5
$\bar{x}$	57.4	53.5	28.2	47.7	46.7

Differences between values with the same letters are non-significant ($P < 0.05$).

3.2. Fresh vegetables

The concentrations of total glucosinolates (GLS) varied significantly ($P < 0.05$) and were dependent on the species of cruciferous vegetables (Table 1). The lowest concentrations GLS were found in white and green cauliflowers (34.1 and 38.1 mg/100 g, respectively) while the highest concentration of GLS was observed in Brussels sprouts (128 mg/100 g). Total GLS content in broccoli and curly cale was intermediate (60.1 and 84.5 mg/100 g, respectively). The obtained concentrations of total GLS are consistent with those reported in similar studies (Ciska et al., 2000; McNaughton & Marks, 2003; Song & Thornalley, 2007).

The concentrations of aliphatic and indole GLS were also variable. The highest concentrations of these GLS were recorded in Brussels sprouts (80.0 and 47.7 mg/100 g of fresh material, respectively). In contrast, the lowest concentration of aliphatic glucosinolates was found in white cauliflower (18.7 mg/100 g of fresh material) and the lowest concentration of indole glucosinolates was in green cauliflower and broccoli (9.5 mg/100 g of fresh material) (Table 2). The relative contribution of aliphatic GLS to total GLS varied from 45.4% (white cauliflower) to 83.5% (broccoli and curly cale). Conversely, the indole GLS accounted for 16.0% of total GLS in broccoli and curly cale whereas, in white cauliflower, they accounted for as much as 54.9% of total GLS (Table 2).

Several break-down products of aliphatic GLS, notably sulforaphane (derived from glucoraphanin), present mainly in broccoli and sinigrin found mainly in Brussels sprouts, are considered to reduce the risk of cancer at several sites. The same is true for break-down products of indole GLS, notably glucobrassicin (Lynn, Collins, Fuller, Hillman, & Ratcliffe, 2006; Smith, Lund, & Johnson, 1998; Zhang et al., 1992).

In these studies, the highest content of glucoraphanin (reaching 97.1% of total aliphatic GLS) was found in broccoli. Much lower values of this contribution were noted for green cauliflower and Brussels sprouts (59.8 and 33.3%, respectively). The relative concentrations of glucoraphanin in other vegetables under study did not exceed 7.0% of total aliphatic GLS. Sinigrin contributions to total aliphatic GLS were 38.0, 28.9 and 20.1%, in Brussels sprouts, white cauliflower and curly cale, respectively. It was not detected in the remaining two species of vegetables under study (Table 2). The highest contributions of glucobrassicin to total indole GLS were found for white cauliflower (80.4%), Brussels sprouts (75.6%), and curly cale (70.9%). The values of the above contributions, for green cauliflower and broccoli, were 60.0% and 48.9%, respectively.

In general, broccoli was the richest source of glucoraphanin (48.7 mg/100 g of fresh material; 81.8% of total GLS). Sinigrin and glucobrassicin were most abundant in Brussel sprouts (30.4 and 36.0 mg/100 g of fresh material; 23.7% and 28.1% of total GLS, respectively) (Table 2). Similarly, highest concentrations of glucoraphanin were observed in broccoli and sinigrin in Brassels sprouts and cauliflower (Song & Thornalley, 2007).

The contribution of aralkyl glucosinolates to total GLS present in the vegetables under study varied from trace amounts in white cauliflower to 1.0% in curly cale (Table 2).

3.3. Blanched and boiled vegetables

Total GLS concentrations in the blanched vegetables were significantly ($P < 0.05$) decreased. These reductions ranged from 2.7% in green cauliflower, through 13.0% in white cauliflower, to 21.1% in curly cale and 30.0% in Brussels sprouts and broccoli (Table 1). More drastic losses of total GLS were found in the boiled vegetables, and ranged from 35.3% in white cauliflower to 72.4% in curly cale (Table 1).

Individual bioactive compounds of the studied vegetables were also affected by blanching and boiling. The losses of glucoraphanin resulting from blanching and boiling of broccoli were 39.1% and 60.6%, respectively. Similarly, blanching and boiling of Brussels sprouts, resulted in significant losses of sinigrin (25.4% and 58.6%, respectively) and glucobrassicin (22.3% and 72.8%, respectively).

The above results may by explained by the decomposition of GLS during various treatments, including cutting, blanching and boiling (De Vos & Blijleven, 1988; McNaughton & Marks, 2003; Verkerk, Van der Gaag, Dekker, & Jongen, 1997). In the initial processes, such as cutting, myrosinase catalyzes decomposition of glucosinolates to release different breakdown products. Generally, aliphatic GLS decompose into volatile isothiocyanates (responsible for pungent taste), whereas indole GLS form indole compounds that are inhibitors of carcinogenesis. Thermal treatments (notably boiling) of cruciferous vegetables lead to inactivation of myrosinase and partial decomposition of thermolabile GLS, especially those of the indole type. In addition, considerable losses of GLS during boiling result from their diffusion into water (Davidek, Velisek, & Pokorny, 1990; Schöne et al., 1994). It can be concluded that during boiling of cruciferous vegetables, aliphatic GLS are generally more stable than are indole GLS, thus indicating that the relative stabilities of individual GLS may be a function of their respective chemical structures.

Table 2
Variations in glucosinolate (GLS) contents in cabbage vegetables exposed to various technological processes (mg/100 g)

	Dry mass	Aliphatic									Aralkyl	Indole			
		Glucoiberin	Progoitrin	Sinigrin	Glucoraphanin	Glucoapoleiferin	Glucoalyssin	Gluconapin	Glucoibervirin	Glucerucin	Gluconastrurtiin	4-Hydroxy-glucobrassicin	Gluco-brassicin	4-Methoxy-glucobrassicin	Neogluco-brassicin
Raw brussels sprouts	14.9	13.3	18.3	30.4	2.66	1.99	2.82	9.63	tr	0.50	0.83	5.98	36.0	5.65	tr
Blanched brussels sprouts	13.6	8.53	15.7	22.7	2.00	1.20	0.80	6.40	tr	0.40	0.66	3.07	28.0	2.67	tr
Boiled brussels sprouts	12.2	5.36	6.74	12.6	1.53	1.84	1.23	3.22	tr	tr	tr	1.23	9.8	3.53	tr
Frozen brussels sprouts	11.4	4.82	6.22	11.3	1.40	1.52	1.02	2.92	tr	0.38	tr	1.02	11.3	3.55	tr
Raw white cauliflower	7.8	2.50	1.52	4.46	0.27	1.43	tr	tr	5.08	0.18	tr	1.43	14.9	1.25	0.98
Blanched white cauliflower	7.1	2.26	3.07	3.79	0.32	1.37	0.56	tr	3.71	tr	tr	1.13	11.6	1.05	0.81
Boiled white cauliflower	7.0	1.71	1.94	3.10	0.23	1.32	0.54	tr	3.18	tr	tr	0.78	7.91	0.85	0.47
Frozen white cauliflower	7.6	2.30	3.24	4.00	0.34	1.96	0.77	tr	4.00	tr	tr	1.19	11.6	1.11	0.68
Raw grren cauliflower	9.6	9.03	0.74	nd	15.9	0.94	tr	tr	1.15	tr	0.31	1.47	5.98	0.73	1.78
Blanched green cauliflower	9.2	6.42	2.34	nd	12.8	1.73	0.82	tr	0.71	tr	0.31	3.36	5.91	1.22	1.43
Boiled green cauliflower	8.3	4.74	1.34	nd	7.87	1.16	0.45	tr	0.54	tr	tr	1.43	2.86	0.54	0.98
Frozen green cauliflower	9.5	8.60	1.55	nd	15.0	1.35	0.41	tr	1.24	tr	0.21	5.08	8.08	1.24	2.07
Raw broccoli	9.6	0.42	tr	tr	48.7	0.52	0.52	tr	tr	tr	0,31	0.31	4.69	2.29	2.29
Blanched broccoli	9.5	0.21	0.74	nd	30.1	1.06	0.32	tr	tr	tr	tr	1.06	4.02	2.64	1.59
Boiled broccoli	7.5	tr	0.54	nd	19.2	0.86	0.24	tr	tr	tr	tr	0.47	2.59	1.33	1.18
Frozen broccoli	9.3	0.21	0.82	nd	37.8	1.34	0.51	tr	tr	tr	tr	1.54	5.66	3.29	2.57
Raw curly cale	18.8	47.1	1.62	14.2	5.05	0.81	tr	0.40	0.81	0.61	0.81	0.61	9.50	2.02	1.01
Blanched curly cale	12.6	38.2	1.21	10.1	4.31	1.35	tr	0.27	0.67	0.40	0.27	1.21	7.00	1.21	0.40
Boiled curly cale	9.1	13.9	0.78	3.71	1.27	1.37	tr	tr	0.20	tr	0.20	tr	1.76	0.20	tr
Frozen curly cale	12.5	36.2	1.21	9.52	4.16	1.21	tr	0.40	0.40	0.40	0.27	1.07	7.24	1.07	0.40

tr – trace <0.05 mg/g dm., nd – not detected.

Moreover, the losses of indole GLS during boiling (resulting from leaching), are five times higher than those of aliphatic GLS (Rungapamestry, Duncan, Fuller, & Ratcliffe, 2007). According to Dekker et al. (2000), the losses of GLS during boiling depend on time, amount, and initial temperature of water used for boiling. For example, it was reported that adding broccoli to cold water, before bringing to boiling, leads to higher losses of GLS, compared to the losses of GLS occuring after direct adding of broccoli to boiling water (Rungapamestry et al., 2007). Also, the diffusion of GLS into water depends rather on the amount of water used for boiling than on time of this treatment (Dekker et al., 2000).

It was also reported that inactivation of myrosinase in cruciferous vegetables, as an effect of boiling, may limit GLS decomposition, thus resulting in consumption of GLS instead of their break-down products by humans (Jiao, Yu, & Habnkin, 1998; Rungapamestry et al., 2007).

In view of the above, the losses of GLS reported in this paper can be related to the processing methods applied. Morever, these results are comparable to the losses of GLS reported earlier and ranging from 25% to 77% (De Vos & Blijleven, 1988; McNaughton & Marks, 2003; Song & Thornalley, 2007; Verkerk et al., 1997).

3.4. Frozen vegetables

Prolonged freezing (48 h, −22 °C) of the blanched vegetables did not produce consistent changes in the concentrations of total GLS. When related to the content of total GLS in the blanched vegetables, the losses of total GLS in frozen Brussels sprouts amounted to 50.7%, and were only 4.5%, but also significant ($P < 0.05$), in frozen curly cale. In contrast, the concentration of total GLS was significantly ($P < 0.05$) increased in frozen green cauliflower and broccoli (20.9% and 28.5%, respectively) (Table 1).

Glucoraphanin concentration in frozen broccoli was not much different, by this treatment, from blanched broccoli (Table 2). On the other hand, the concentrations of sinigrin and glucobrassicin in frozen Brussels sprouts were significantly decreased ($P < 0.05$) by 49.8% and 40.3%, respectively.

Much greater losses of GLS, resulting from prolonged freezing of cruciferous vegetables, were reported by Song and Thornalley (2007). However, in their experiment, the vegetables were stored at much lower temperature (−85 °C) and much longer (two months), and without prior blanching. Indeed, the resulting losses of total GLS were 33% and those of individual GLS were 10–53%, and were much higher than those occurring in a refrigerator (at 4–8 °C).

The above losses of GLS may be related to disruption of cell plants by ice crystals during freezing. Consequently, after thawing and myrosinase activation, GLS may undergo almost complete hydrolysis to their breakdown products. This process, usually combined with thermal treatment (e.g. boiling), may lead to very high losses of GLS (Davidek et al., 1990).

The above mechanism might explain the losses of total GLS, sinigrin and glucobrassicin in frozen Brussels sprouts. On the other hand, negligible losses of total GLS in frozen curly cale, and even increased concentrations of these compounds in frozen green cauliflower and broccoli, are difficult to interpret. The possible explanation is that frozen vegetables, examined in this paper, were kept frozen (48 h, −22 °C) and directly freeze-dried prior to analysis. This might have minimized the activity of myrosinase in frozen green cauliflower and broccoli. At the same time, we cannot offer an explanation for increased concentrations of total GLS in frozen green cauliflower and broccoli.

4. Conclusions

1. Among the studied cruciferous vegetables, Brussels sprouts are the richest source of aliphatic and indole GLS. The lowest concentrations of aliphatic GLS are found in white cauliflower, whereas green cauliflower and broccoli are poor sources of indole GLS.
2. Among biologically active individual GLS, glucoraphanin is most abundant in broccoli, while sinigrin and glucobrassicin are abundant in Brussels sprouts.
3. Blanching of cruciferous vegetables results in significant decreases in total GLS, ranging from 2.7% (green cauliflower) to 30.0% (Brussels sprouts and broccoli).
4. Boiling of cruciferous vegetables leads to high losses of total GLS, compared to blanching. These losses range from 35.3% (white cauliflower) to 72.4% (curly cale).
5. Prolonged freezing (48 h) of cruciferous vegetables produced opposite changes (either increases or decreases) in concentrations of GLS.

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