Comparison of Sous-vide methods and traditional hydrothermal treatment on GLS content in *Brassica* vegetables

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Abstract Brassica vegetables are a source of many biologically active compounds, including glucosinolates (GLS). At present, GLS attract a special attention because of their healthy properties. Four *Brassica* vegetables: cauliflower, Romanesco-type cauliflower, Brussels sprouts, and broccoli were examined. Analyses of GLS were done in raw, sous-vide cooked, traditionally cooked, and steamed vegetables as well as in samples stored for 5 days at 2 ± 1 °C. In the examined vegetables, 9 GLS were identified. The content of vitamin C was also determined. Moreover, microbiological analyses were performed. Among the investigated vegetables, the highest content of GLS was observed in the steamed samples. The traditional cooking of broccoli and Romanesco-type cauliflower causes a significant decrease of GLS as compared to the raw materials. The same explicit tendency was also observed in the case of cooking by the sous-vide method of Romanesco-type cauliflower and Brussels sprouts. Brussels sprouts, cooked by the sous-vide method, contained almost 40% less GLS than those cooked in water. Storage of the treated vegetables at 2 ± 1 °C led to a further reduction of the GLS level. It must be emphasized that the highest losses were observed in broccoli after sous-vide cooking (over 30%).

Our results indicate that sous-vide method can be an advantageous technique of processing broccoli destined only for direct consumption.

Keywords Sous-vide method · Cooking · Steaming · Glucosinolates · Brussels sprouts · Cauliflower

Introduction

GLS (GLS) are sulfur- and nitrogen-containing plant secondary metabolites common in *Brassica* vegetables [1]. The characteristic flavour and odour of these vegetables are the result of the presence of GLS. GLS and their breakdown products are of particular interest in food research because of their anticarcinogenic properties [2]. A number of epidemiological studies have identified an inverse association between the consumption of *Brassica* vegetables and the risk of colon and rectal cancer [3]. The GLS are not biologically active themselves, but only their enzymatic derivatives are [4]. The products of GLS breakdown, particularly the aromatic and indole products, have been shown to act as anticarcinogens by inhibition of the phase I enzymes and induction of the phase II enzymes that affect the xenobiotic transformations. Similar effects are also described for some degradation products of the aliphatic GLS [5]. This is why nutritional recommendations suggest an increase in the total consumption of *Brassica* vegetables, such as cabbage, Brussels sprouts, cauliflower, broccoli, etc.

In the *Brassicaceae* family, the plant's genetic background is the major factor determining GLS concentration and composition, although environmental conditions and physiological factors also influence GLS expression and accumulation [3]. On the other hand, many steps in the food production chain, such as processing and preparation

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of vegetables as well as the preservation method, may have an impact on the levels of GLS [4, 6]. Most GLS are relatively stable at high temperatures, but are easily enzymatically hydrolyzed by the enzyme—myrosinase. This enzyme is present in the *Brassica* vegetables' tissues and is released into the plant cells due to damage, for example, during the technological processes [7]. Several studies [8–11] are concerned on the methods of GLS extracting from raw plant materials to obtain the highest possible amount of these compounds in the sample. The inactivation of myrosinase is one of the crucial issues. Meanwhile, pretreatment—being a necessary step in technological processing of vegetables—could cause mechanical damage (physical injury) and thus led to the activation of myrosinase. According to Oerlemans and Barrett [2], also during thermal processing of *Brassica* vegetables, the GLS levels can be reduced because of several mechanisms: enzymatic breakdown, thermal breakdown, and leaching into the heating medium. Cooking at high temperatures, denatures myrosinase in the vegetable material, resulting in a lower conversion of GLS to isothiocyanates when chewed. GLS of consumed vegetables are degraded to isothiocyanates (ITC) by myrosinase of the gut bacteria [12]. Angelino et al. [13] suggest that plant myrosinase is essential for an effective dose of ITC to be formed and absorbed, supporting the traditional idea that cooked *Brassica* are less likely to provide health benefits than uncooked vegetables. In the study performed by Vermeulen et al. [14], plasma sulforaphane and metabolites in those eating cooked broccoli were one-tenth that of those eating raw broccoli.

According to Cieřlik et al. [15], the traditional cooking (raw or frozen) of *Brassica* vegetables resulted in a significant reduction of GLS. The sous-vide (SV) cooking method is one of the many variants of the cook and chill catering technology. It is nowadays employed to process convenience foods, including ready meals. It consists in cooking of highly nutritious raw materials (e.g., vegetables) at product-specific temperatures and time in vacuum pouches, followed by rapid chilling and low-temperature storage (0–3 °C) [16]. This method improves sensory quality (i.e., texture, tenderness, and flavour) as well as preserves the nutritional status more than conventional cooking. In the literature, little is known about the influence of vegetable cooking under low temperature and after vacuum packaging on the level of GLS in the final products.

Taking into consideration growing popularity of SV method, the knowledge of its effect on biological important dietary components is truly needed but not only from methodological but also from consumer point of view (the real GLS amount consumed with the vegetables after all treatments—ready to eat products). For that reason, the aim was to compare the effect of sous-vide cooking vs traditional cooking or steaming, on the GLS content in selected

Brassica vegetables. To make this research more distinctive from other of this type and having regard to important role of vitamin C in activation of myrosinase, the level of this compounds in raw materials was also determined. Moreover, to ensure consumer safety, microbiological quality of processed vegetables was assessed.

Materials and methods

Material

Four common *Brassica* vegetables: cauliflowers (*Brassica oleracea* var. *botrytis*),—white rose and Romanesco-type cauliflower—green rose, Brussels sprouts (*Brassica oleracea* var. *gemmifera*), and broccoli (*Brassica oleracea* var. *botrytis italica*) were investigated. The vegetables were bought in a local supermarket (Krakow, Poland) in 2014. The batch of each vegetable (5 kg green mass, in triplicate) was cut vertically (into four or eight pieces, depending on their size), and then divided into four sub-samples, which were subsequently used as raw ($n=3$), cooked by the sous-vide method ($n=3$), “traditionally”—in water (at 500:1500 ratio of vegetables to water, m/v), cooked ($n=3$), and steamed ($n=3$). Raw vegetables were washed under running water, dried on filter paper, divided into florets (excluding Brussels sprouts), and then frozen at -22 ± 1 °C. After freezing, this sub-sample was immediately freeze-dried using a Christ Alpha 1–4 apparatus (Christ, Germany), and ground in Tecator Knifetec 1095 Sample Mill (Tecator, Sweden), until uniform powder was obtained. The sous-vide cooking was performed using a sous-vide system—model 225,448 (Hendi, Germany), at 90 °C for 45 min (cauliflowers and broccoli) or 50 min (Brussels sprouts). This process was preceded by vacuum packaging in VBN-4 (RM Gastro, Czech Republic). The traditional cooking of vegetables was carried out in unsalted water for 10 (cauliflowers and broccoli) or 15 min (Brussels sprouts), in a stainless steel pot, on the induction stove (Hendi, Germany). Sample steaming was performed using Combi-Steam Oven model 225,547 (Hendi, Germany) at 100 °C for 7 min. On the basis of the results of preliminary studies, one timepoint for each treatment was chosen. The main criterium was organoleptic characteristic of the final product (consumer acceptance).

After each thermal treatment, the vegetables were immediately chilled in RedFox SHS-511 shock cooling chamber (RM Gastro, Czech Republic) and stored at a controlled temperature of 2 ± 1 °C for 5 days. The preparation of samples after thermal treatment and storage for the analyses was performed by the same procedure as used for raw vegetables.

Chemical analysis

The content of dry matter was determined by gravimetric methods according to Polish standard [17] and expressed in g per 100 g. The analysis of vitamin C (L-ascorbic acid) content was performed by the colorimetric method, according to Megazyme assay procedure K-ASCO 01/14. Approximately 10 g of vegetables were homogenized with 10 mL of 1 M potassium phosphate buffer (pH 3.5) for 1 min. Then, the pH was adjusted to 3.5–4.0 with 2 M KOH and the mixture was quantitatively transferred to a 500 mL volumetric flask, filled in using distilled water, mixed, and filtered. The calculation of L-ascorbic acid content was done by the use of Megazyme Mega-CalTM, downloaded from the Megazyme website (<http://www.megazyme.com>), and the results were expressed in mg per 100 g wet weight. On the basis of the differences between initial (before process) and final (after process) mass of vegetables, process efficiency (%) was calculated.

Analyses of GLS (GLS) were done in raw, sous-vide cooked, traditionally cooked (in water), and steamed vegetables as well as in the samples stored for 5 days at a controlled temperature of 2 ± 1 °C. GLS were extracted from the research materials according to the method reported in the Official Journal of European Communities [18]. Briefly, duplicate 200 mg samples of freeze-dried material were extracted thrice with cooking 70% methanol. Since all the examined vegetables 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 chromatographic analysis of GLS was carried out according to the modified method of Heaney et al. [19]. Desulpho-GLS were separated on the HPLC system with an auto injector, Spherisorb ODS-2 3 Micron column (150 m $\times$ 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, isocratically 99% B for 6 min, linear gradient to 1% B for 5 min, and 1% B for 8 min. GLS were detected at $k = 229$ nm. The content of GLS was quantified using the internal standard as well as relevant relative response factors [18], and expressed in μmol per 100 g of fresh weight.

Microbiological analysis

The microbiological status of cooked (all methods) vegetables as well as the samples after storage was analyzed immediately after the process. The fresh vegetables were analyzed in the day of purchase.

To determine the total count of microorganisms, the horizontal method was used, according to PN-EN ISO 4833-1:2013-12E [20]. The enumeration of coliforms

was performed on the basis of PN-ISO 4832/2007 [21], *Listeria monocytogenes* according to PN-EN ISO 11290-2/2000 [22] and anaerobic bacteria according to PN-ISO 15213:2005P [23].

Statistical analysis

All technological treatments and analysis were done in triplicate. The results were expressed as mean values $\pm$ SD (standard deviation). The data were subjected to two-factorial (4×3) analysis of variance, including four species of *Brassica* vegetables and three processing methods. The differences between the means were evaluated using the post-hoc NIR Fisher test, with the significance level established at $\alpha < 0.05$. Statistica 10.1 software package (StatSoft Inc., USA) was used for statistical analysis.

Results and discussion

Dry matter content and microbiological quality of *Brassica* vegetables: raw and after different cooking treatments

It was shown that the dry matter levels in raw cauliflower, broccoli, Romanesco-type cauliflower, and Brussels sprouts were 8.42, 12.81, 13.95, and 15.05 g/100 g, respectively (Table 1). Similar results for cauliflower (7.8 g/100 g) and Brussels sprouts (14.9 g/100 g) as well as lower for Romanesco-type cauliflower (9.6 g/100 g) and broccoli (9.6 g/100 g) were obtained by Cieřlik et al. [15]. A higher dry matter content in cauliflower was determined by Ahmed and Ali [24]. In turn, Gębczyński and Lisiewska [25] reported 10.34 g/100 g of the dry matter in broccoli. These differences may be due either to the species or the varietal diversification or different cultivation conditions. The traditional cooking of all the examined vegetables led to a statistically significant decrease in the dry matter amount. Such observations concerning *Brassica* vegetables were made also by other authors [15, 24, 26]. Steamed vegetables had the highest content of dry matter. The increase of this parameter in Brussels sprouts var. Ajax, as compared to raw vegetable, was shown by Podędek et al. [27]. The results regarding the dry matter levels in raw vegetables as well as these subjected to thermal processing are in line with the values relating to the process efficiency. The highest efficiency was obtained for the traditional cooking, indicating that the increase in weight of the cooked vegetables was due to water absorption, dilution of their constituents, and finally the reduction of dry matter content in the finished product. The minimal (from 0.6 to 6.7%) real losses of dry matter (having regard to the process efficiency) in vegetables subjected to cooking by the sous-vide method

Table 1 Content of dry matter and the efficiency of vegetable thermal processing

Vegetable	Sample treatment	Dry matter (g/100 g)	Efficiency of process (%)
Cauliflower	Raw	8.42b ± 0.03	–
	Sous vide	8.22b ± 0.03	98.0
	Cooking	7.80a ± 0.34	101.0
	Steaming	8.67b ± 0.43	95.0
Broccoli	Raw	12.81b ± 0.27	–
	Sous vide	12.85b ± 0.51	98.0
	Cooking	10.89a ± 0.26	102.7
	Steaming	14.25c ± 0.39	89.7
Romanesco-type cauliflower	Raw	13.95b ± 0.59	–
	Sous vide	14.01c ± 0.39	99.0
	Cooking	12.63a ± 0.15	104.3
	Steaming	14.89c ± 0.61	91.8
Brussels sprouts	Raw	15.05c ± 0.37	–
	Sous vide	14.25b ± 0.09	98.5
	Cooking	13.40a ± 0.40	102.3
	Steaming	15.40c ± 0.74	94.0

The values of dry matter content are expressed as mean ± SD

The values marked with the same letters not differ significantly at $p > 0.05$

were observed, and the reason could be primarily the previous vacuum packaging (thus preventing direct contact of raw material with water), and also a lower temperature of the process as compared to the traditional cooking.

The raw materials were characterized by total microbial counts from 4.4×10^3 cfu/g (cauliflower—white rose) to 8.9×10^6 cfu/g (broccoli). In all fresh vegetables, *Listeria monocytogenes* was present (6.7×10^1 – 9.0×10^2). Moreover, in the case of Romanesco-type cauliflower (3.0×10^3) as well as broccoli (8.5×10^2), coliforms were detected. The analysis of microbiological quality did not show the presence of *Listeria monocytogenes*, anaerobic bacteria, and coliforms, neither immediately after the process, nor after 5 days of storage at 2 ± 1 °C. The total microbial count in steamed vegetables varied from 6.0×10^1 to 8.5×10^1 cfu/g, and in traditionally cooked vegetables—from 2.5×10^1 to 1.6×10^2 cfu/g, whereas in the samples subjected to a sous-vide cooking, it ranged between 2.4×10^2 and 9.8×10^3 cfu/g. After 5 days of storage, an enhancement of the total microbial count was observed, with the highest value obtained for cauliflower (6.4×10^4 cfu/g).

The GLS changes after different cookings of vegetables as compared to raw materials

Nine GLS (GLS), including six aliphatic and three indole, were identified by the HPLC method in the examined *Brassica* vegetables (Table 2). In addition, the total GLS levels were calculated.

GLS content in raw vegetables

Among the evaluated vegetables, Brussels sprouts were characterized by the highest content of GLS ($215.26 \mu\text{mol}/100$ g wet weight). The higher values were obtained by Ciska et al. [28] and Carlson et al. [29], whereas lower—by Song and Thornalley [12]. It was also shown that sinigrin, glucoiberin, and glucobrassicin dominated in this vegetable. Similar tendencies were reported by Ciska et al. [5] as well as Cieřlik et al. [15].

Significantly less GLS were determined in Romanesco-type cauliflower ($133.4 \mu\text{mol}/100$ g wet weight) and broccoli ($119.24 \mu\text{mol}/100$ g wet weight), with the highest amount obtained for glucoraphanin. Meanwhile, sinigrin was not present in Romanesco-type cauliflower (Table 2). These results were in line with those obtained by Cieřlik et al. [15]. Brown et al. [30] reported that the glucoraphanin was dominant GLS in ten genotypes of broccoli grown over different environments. Similar data were obtained by Song and Thornalley [12].

The lowest GLS content was found in white rose cauliflower ($62.25 \mu\text{mol}/100$ g wet weight), as presented in Table 2. Delonga et al. [31] obtained higher amounts of GLS sum, whilst lower values were reported by Mithen et al. [32] as well as Song and Thornalley [12].

GLS are hydrolyzed by the enzyme myrosinase (thio-glucosylhydrolase; E.C.3.2.1.147) to the various compounds (isothiocyanates, nitriles, thiocyanates, epithionitriles, and oxazolidine-2-thiones) [33]. Mithen et al. [32] stated that

Table 2 Content of glucosinolates ($\mu\text{mol}/100$ g wet weight) and ascorbic acid ($\text{mg}/100$ g wet weight) in raw vegetables

Vegetable	Gluciberin	Progoitrin	Sinigrin	Glucoraphanin	Gluconapin	Gluconasturtin	Total aliphatic GLS	Glucobrassicin	4-Methoxyglucobrassicin	Neoglucobrassicin	Total indole GLS	GLS sum	Ascorbic acid
Cauliflower	14.13b $\pm$ 0.008	3.57b $\pm$ 0.005	18.31c $\pm$ 0.012	1.41a $\pm$ 0.001	3.05a $\pm$ 0.009	3.43a $\pm$ 0.003	43.89a $\pm$ 0.040	11.54a $\pm$ 0.009	1.69a $\pm$ 0.005	2.25b $\pm$ 0.000	18.36a $\pm$ 0.009	62.25a $\pm$ 0.05	57.80a $\pm$ 0.18
Broccoli	9.14a $\pm$ 0.005	2.81a $\pm$ 0.005	1.80b $\pm$ 0.001	63.18d $\pm$ 0.130	5.97d $\pm$ 0.005	7.05b $\pm$ 0.008	89.95b $\pm$ 0.165	15.04b $\pm$ 0.025	4.17b $\pm$ 0.009	9.93d $\pm$ 0.002	29.29b $\pm$ 0.041	119.24b $\pm$ 0.37	63.83b $\pm$ 0.40
Romanesco-type cauliflower	19.39c $\pm$ 0.001	4.08c $\pm$ 0.020	0.00a $\pm$ 0.00	53.86c $\pm$ 0.002	5.10c $\pm$ 0.005	5.43b $\pm$ 0.001	87.86c $\pm$ 0.056	11.02c $\pm$ 0.025	3.70b $\pm$ 0.001	5.28c $\pm$ 0.048	20.00a $\pm$ 0.067	133.40c $\pm$ 0.24	56.40a $\pm$ 1.13
Brussels sprouts	43.48d $\pm$ 0.037	29.24d $\pm$ 0.005	72.80d $\pm$ 0.009	9.96b $\pm$ 0.047	3.79b $\pm$ 0.008	20.65c $\pm$ 0.009	179.93d $\pm$ 0.047	29.48d $\pm$ 0.009	5.85c $\pm$ 0.001	0.00a $\pm$ 0.00	35.33c $\pm$ 0.005	215.26d $\pm$ 0.08	98.30c $\pm$ 1.83

Values are expressed as mean $\pm$ SDThe values in the same columns denoted with different letters differ statistically significantly at $p \leq 0.05$

if the breakdown happens as the result of plant-derived myrosinase action, GLS are rapidly hydrolyzed in the proximal part of the intestine. If myrosinase is inactivated (for example, by the thermal treatment) before vegetable consumption, ionized form of GLS enables them to reach distal part of the intestine (when they are metabolized by bacterial enzymes). According to Mithen et al. [32], myrosinase is located in the cytoplasm of specialist myrosin cells scattered throughout the plant tissue and may also be located in the cytoplasm of other cells. Upon mechanical injury of the tissue (e.g., chewing or cutting), the enzyme comes in contact with its substrate and hydrolyzes it [34].

The extent of hydrolysis of GLS and the nature as well as the composition of the breakdown products formed are known to be influenced by various characteristics of the hydrolysis medium. Intrinsic factors, such as coexisting myrosinase and its cofactors ascorbic acid, epithiospecifier protein (ESP), or ferrous ions and extrinsic factors, such as pH and temperature, can affect the hydrolysis of GLS [35].

Among the examined vegetables, the highest content of ascorbic acid was found in Brussels sprouts, and the lowest—in Romanesco-type cauliflower (Table 2). The high level of vitamin C (98.3 mg/100 g) in raw Brussels sprouts could contribute to enhanced myrosinase activity and GLS breakdown. There was 215.26 μmol GLS/100 g wet weight in fresh vegetables and up to 330.91 $\mu\text{mol}/100$ g wet weight in steamed samples (thermal inactivation of myrosinase)—Table 3.

There are evidences that the content of GLS in fresh vegetables can be underestimated due to the methodological limitations [8, 28]. For this reason, it is difficult to evaluate the real initial value of GLS when regarding the effect of different technological processes on their amount. For example, Verkerk and Dekker [36] proposed the subjection of plant material to short-term thermal processing (cooking in water or microwaving) before GLS determination. In their opinion, GLS are better extracted from cooked vegetables than from raw materials. Vallejo et al. [37] showed that steaming of broccoli for 3.5 min causes a minimal reduction of GLS, confirming the efficacy of this method in the inactivation of endogenous myrosinase.

Rungapamestry et al. [35] demonstrated that steaming for 420 s leads to over a 90% reduction of this enzyme. Our results confirmed that steamed vegetables were characterized by the highest extractable GLS content. It is to emphasize that the method applied to vegetable processing was using the apparatus with forced convection, enabling additional enhancement of heat transfer coefficient, and in consequence possibly fast increase in the temperature inside the product.

Our study showed that steaming of vegetables resulted in a significant enhancement of GLS content as compared to fresh vegetables. Particular importance attaches the level

Table 3 Content of glucosinolates in the vegetables immediately after thermal treatment (umol/100 g wet weight)

Vegetable	Thermal treatment	Glucoiberin	Progoitrin	Sinigrin	Glucoaraphanin	Gluconapin	Gluconasturtin	Total aliphatic GLS	Glucobrassicin	4-Methoxyglukobrassicin	Neoglucobrassicin	Total indole GLS	GLS sum
Cauliflower	Steaming	52.77b ± 0.027	10.42c ± 0.016	51.98c ± 0.006	2.48c ± 0.009	12.81c ± 0.005	11.17c ± 0.007	141.63c ± 0.003	18.92c ± 0.003	2.12c ± 0.001	3.26c ± 0.015	36.02c ± 0.009	163.44b ± 0.041
	Cooking	33.84a ± 0.319	6.61a ± 0.031	28.86b ± 0.015	1.5a9 ± 0.005	3.17a ± 0.005	6.04b ± 0.008	80.12a ± 1.048	14.01b ± 0.111	1.93b ± 0.009	2.91b ± 0.0023	23.62b ± 0.020	103.74a ± 0.779
	Sous vide	50.89b ± 0.013	7.51b ± 0.006	23.37a ± 0.005	2.1b2 ± 0.003	3.75b ± 0.001	1.96a ± 0.009	88.71b ± 0.207	9.68a ± 0.003	1.50a ± 0.001	2.06a ± 0.045	15.24a ± 0.021	103.95a ± 0.096
Broccoli	Steaming	44.05c ± 0.086	10.49c ± 0.013	0.00	152.77c ± 0.056	18.23c ± 0.018	10.74c ± 0.002	204.20c ± 0.320	19.10c ± 0.009	4.34c ± 0.001	12.37c ± 0.089	35.81c ± 0.002	239.65c ± 0.373
	Cooking	6.27a ± 0.010	3.51a ± 0.002	0.00	61.09a ± 0.010	2.64a ± 0.010	4.57b ± 0.007	78.08a ± 0.070	6.21a ± 0.018	2.05a ± 0.009	2.69a ± 0.045	10.95a ± 0.025	89.03a ± 0.060
	Sous vide	16.98b ± 0.014	5.85b ± 0.002	0.00	127.02b ± 0.000	5.13b ± 0.001	4.41a ± 0.001	159.39b ± 0.009	13.11b ± 0.024	3.98b ± 0.001	4.58b ± 0.045	21.67b ± 0.041	213.51b ± 0.075
Romanesco cauliflower	Steaming	37.08b ± 0.029	8.11b ± 0.002	5.42b ± 0.008	69.60c ± 0.010	12.91c ± 0.009	15.23c ± 0.002	148.35c ± 0.173	22.53c ± 0.027	6.28c ± 0.008	6.67b ± 0.005	35.48c ± 0.038	183.83c ± 0.048
	Cooking	18.56a ± 0.001	7.30a ± 0.083	3.23a ± 0.005	38.87b ± 0.012	4.22b ± 0.002	9.46a ± 0.002	81.64b ± 0.031	19.26b ± 0.010	5.66b ± 0.001	6.91b ± 0.009	31.83b ± 0.035	113.47b ± 0.040
	Sous vide	19.06a ± 0.009	10.32c ± 0.014	0.00	37.64a ± 0.036	2.02a ± 0.008	10.85b ± 0.008	79.89a ± 0.045	15.09a ± 0.035	4.17a ± 0.009	4.09a ± 0.002	23.35a ± 0.005	103.24a ± 0.020
Brussels sprouts	Steaming	73.29c ± 0.005	48.27c ± 0.001	114.82c ± 0.002	13.10c ± 0.009	9.75c ± 0.002	26.27dc ± 0.001	285.50c ± 0.001	38.83c ± 0.001	6.58c ± 0.015	0.00	45.41c ± 0.015	330.91c ± 0.021
	Cooking	57.92b ± 0.013	42.44b ± 0.025	109.89b ± 0.005	9.69b ± 0.018	3.25a ± 0.005	25.16b ± 0.018	253.29b ± 0.005	36.27b ± 0.009	5.96b ± 0.018	0.00	42.23b ± 0.027	295.52b ± 0.055
	Sous vide	42.62a ± 0.036	41.00a ± 0.009	71.00a ± 0.014	6.37a ± 0.001	4.87b ± 0.009	9.75a ± 0.009	175.61a ± 0.269	16.67a ± 0.009	2.99a ± 0.008	0.00	19.66a ± 0.009	195.27a ± 0.376

Values are expressed as mean ± SD

The values in the same columns denoted with different letters differ significantly at $p \leq 0.05$

of GLS, mainly glucoiberin and sinigrin, in cauliflower. This vegetable, subjected to steaming, contained over 2.5-fold higher amount of GLS than raw cauliflower. Twice as much GLS, in particular aliphatic, were determined also in the case of steamed broccoli. The subjection of Brussels sprouts to this process led to 1.53-fold increase in GLS. According to the study of Ciska et al. [28], the initial value (being the sum of GLS in cooked Brussel sprouts and in the water left after the process) was 1.37-fold higher, compared with fresh vegetable. Miglio et al. [38] showed higher concentration of GLS in steamed broccoli (93.4 $\mu\text{mol/g d.m.}$), in comparison with raw (71.4 $\mu\text{mol/g d.m.}$) and traditionally cooked vegetable (29.3 $\mu\text{mol/g d.m.}$).

In light of the obtained results, as well as literature data, when discussing the effect of other technological processes, the level of GLS obtained for steamed vegetables was taken as the initial value of their content.

In our opinion, it is all the more justified that in most vegetables, this level was stable also during storage at 2 ± 1 °C of ready to eat products. After 5 days of storage, steamed cauliflower and broccoli contained over 95% of GLS value determined immediately after the process (Table 4). Although in the case of Romanesco-type cauliflower as well as Brussels sprouts, this amount was lower; it was, however, over 80% initial value.

Comparison of different cooking methods (traditional cooking, sous vide, and steaming) on GLS content

Brassica vegetables, besides beneficial health effects, can exert toxic and goitrogenic action. Nonetheless, as most of vegetables that are abundant in 2-hydroxyalkenyl GLS are cooked before consumption, the goitrogenic action should not have been counted [39]. The study performed by Verkerk [40] showed the reduction (40–70%) in GLS content during cooking, which was mainly caused by the transition of these compounds to water. As a result, it was recognized that microwave cooking (in view of small amount of necessary water) would be a better method of vegetable processing. Meanwhile, this method leads not only to high losses of vitamin C, but also over a 70% reduction of GLS [cit. from 2].

In our opinion, the sous-vide method can be a considerable alternative of cooking. This method—because of barrier heating (earlier vacuum packaging)—makes impossible the direct contact between raw material and water environment. From the other side, it is characterized by a lower range of the used temperatures.

Sous-vide cooking of broccoli led to higher GLS content in comparison with traditionally cooked vegetables as well as similar to steamed broccoli (Table 3). Among the analyzed compounds, glucoraphanin, glucoiberin, progoinin, and glukonapin were in higher amounts

(almost twofold) in broccoli prepared by the sous-vide method, comparing to vegetable cooked traditionally in the water. As regards indole GLS, it was twice more glucobrassicin than 4-metoxylucobrassicin. Differences in thermal the breakdown of GLS can be the result of the presence of specific plant components, which negatively influence the GLS stability. The higher content of GLS in plant tissues, the higher it will be their susceptibility to breakdown [4]. It was shown that the total content of GLS was similar in cooked and SV-treated cauliflower, with higher amount of indole GLS (especially glucobrassicin) in traditionally (in water) cooked vegetable and lower level of aliphatic GLS (mainly glucoiberin) in relation to cauliflower prepared by the sous-vide method.

Cooking of Brussels sprouts and Romanesco-type cauliflower by the sous-vide method resulted in much higher losses of GLS, both indole and aliphatic, in comparison with the traditional cooking. It is to emphasize that the use of this technique of processing was particularly disadvantageous and reduced the total content of GLS, also in relation to raw vegetable. Simultaneously, a slight increase was observed in the amount of indole GLS (glucobrassicin and 4-metoxylucobrassicin) in Romanesco-type cauliflower was observed. Kapusta-Duch et al. [4] reported that cooking causes a significant decrease in the content of GLS, comparing it with raw vegetables. These authors observed that indole GLS were more susceptible to processing, and their losses gained over 90% (mainly glucobrassicin). Added to that is the fact that sinigrin was not identified in sous-vide cooked Romanesco-type cauliflower, whereas this compound was present both in traditionally cooked and steamed vegetables. Myrosinase inactivation in *Brassica* vegetables, as the effect of cooking, can reduce GLS breakdown, which results in GLS consumption (instead the products of their breakdown). It is known that GLS can be hydrolyzed not only by myrosinase, but they are also degraded by temperature as well as reduced to isothiocyanates by gut microflora [41]. It is to emphasize that the temperatures during processing by SV method are relatively low. Thus, the thermal degradation of GLS (observed in our study) was probably lower than during traditional cooking. Isothiocyanates are responsible for the reduction in the risk of many cancers [6]. Anticancer properties of these compounds arise from inhibition of phase I enzymes (the role in carcinogen bioactivation) and induction of phase II enzymes [41]. According to Sarvan et al. [41], an optimal technological process should allow to preserve GLS in the unchanged form.

Brussels sprouts, cooked by the sous-vide method, contained almost 40% less GLS than cooked in water. The exception was gluconapin, with a 50% higher content in the samples after sous-vide cooking. As Sarvan et al. [34]

Table 4 Content of glucosinolates in the vegetables after 5 days of storage ($\mu\text{mol}/100\text{ g}$ wet weight)

Vegetable	Thermal treatment	Glucobrassicin	Progoitrin	Sinigrin	Glucoraphanin	Gluconapin	Gluconasturtin	Total aliphatic GLS	Glucobrassicin	4-Methoxyglucobrassicin	Neoglucobrassicin	Total indole GLS	GLS sum
Cauliflower	Steaming	51.81c $\pm$ 0.026	10.05c $\pm$ 0.012	51.63c $\pm$ 0.001	2.12c $\pm$ 0.008	9.69b $\pm$ 0.025	10.31c $\pm$ 0.001	135.61c $\pm$ 0.005	16.76c $\pm$ 0.034	1.93b $\pm$ 0.013	3.12b $\pm$ 0.003	21.81b $\pm$ 0.049	157.42c $\pm$ 0.087
	Cooking	33.54a $\pm$ 0.009	6.75a $\pm$ 0.031	28.33b $\pm$ 0.001	1.82b $\pm$ 0.019	3.76a $\pm$ 0.001	6.07b $\pm$ 0.005	80.27a $\pm$ 0.015	13.82b $\pm$ 0.003	2.30c $\pm$ 0.009	3.12b $\pm$ 0.014	45.54c $\pm$ 0.008	125.47b $\pm$ 0.051
	Sous vide	50.33b $\pm$ 0.028	7.30b $\pm$ 0.017	23.84a $\pm$ 0.063	1.38a $\pm$ 0.026	3.42a $\pm$ 0.045	1.16a $\pm$ 0.003	87.43b $\pm$ 0.543	9.66a $\pm$ 0.016	1.15a $\pm$ 0.012	2.15a $\pm$ 0.003	12.96a $\pm$ 0.073	100.39a $\pm$ 1.013
Broccoli	Steaming	36.35c $\pm$ 0.025	8.78c $\pm$ 0.013	0.00	125.55c $\pm$ 0.517	15.71c $\pm$ 0.002	10.38c $\pm$ 0.014	196.87c $\pm$ 0.891	19.35c $\pm$ 0.001	4.07b $\pm$ 0.001	7.78c $\pm$ 0.001	31.20c $\pm$ 0.001	228.07c $\pm$ 0.036
	Cooking	5.26a $\pm$ 0.004	2.78a $\pm$ 0.004	0.00	45.24a $\pm$ 0.015	2.42a $\pm$ 0.002	4.29a $\pm$ 0.004	59.99a $\pm$ 0.021	6.71a $\pm$ 0.011	1.94a $\pm$ 0.014	2.15a $\pm$ 0.002	10.80a $\pm$ 0.021	70.79a $\pm$ 0.084
	Sous vide	16.85b $\pm$ 0.056	5.81b $\pm$ 0.002	0.00	105.37b $\pm$ 0.014	4.83b $\pm$ 0.005	4.50b $\pm$ 0.005	137.36b $\pm$ 0.126	12.22b $\pm$ 0.002	3.89b $\pm$ 0.005	4.01b $\pm$ 0.005	20.12b $\pm$ 0.027	157.48b $\pm$ 0.934
Romanesco-type cauliflower	Steaming	26.72c $\pm$ 0.001	8.85b $\pm$ 0.001	2.02 $\pm$ 0.009	67.49b $\pm$ 0.037	11.60c $\pm$ 0.019	8.68b $\pm$ 0.005	125.36c $\pm$ 0.058	18.44b $\pm$ 0.029	5.71c $\pm$ 0.005	6.28b $\pm$ 0.002	30.43c $\pm$ 0.084	155.79b $\pm$ 0.283
	Cooking	16.92a $\pm$ 0.002	7.44a $\pm$ 0.002	0.00	36.15a $\pm$ 0.024	2.73b $\pm$ 0.018	8.00b $\pm$ 0.002	71.24a $\pm$ 0.060	11.86a $\pm$ 0.012	4.21b $\pm$ 0.002	4.35a $\pm$ 0.002	20.42b $\pm$ 0.040	91.66a $\pm$ 0.199
	Sous vide	19.07b $\pm$ 0.005	8.95b $\pm$ 0.001	0.00	36.62a $\pm$ 0.045	2.11a $\pm$ 0.002	5.46a $\pm$ 0.015	72.31b $\pm$ 0.159	11.45a $\pm$ 0.009	3.13a $\pm$ 0.001	4.36a $\pm$ 0.002	18.94a $\pm$ 0.027	91.25a $\pm$ 0.317
Brussels sprouts	Steaming	53.51c $\pm$ 0.320	42.29c $\pm$ 0.039	102.82b $\pm$ 0.175	13.09c $\pm$ 0.010	7.68c $\pm$ 0.001	18.92b $\pm$ 0.015	238.31c $\pm$ 2.179	32.28b $\pm$ 0.049	5.91b $\pm$ 0.001	0.00	38.19b $\pm$ 0.073	276.50b $\pm$ 3.052
	Cooking	44.37b $\pm$ 0.019	38.41b $\pm$ 0.053	106.46c $\pm$ 0.019	8.71b $\pm$ 0.005	2.38a $\pm$ 0.002	24.86c $\pm$ 0.025	225.19b $\pm$ 0.043	34.69c $\pm$ 0.053	6.63c $\pm$ 0.001	0.00	41.32c $\pm$ 0.064	266.51b $\pm$ 0.211
	Sous vide	38.00a $\pm$ 0.020	28.03a $\pm$ 0.036	71.01a $\pm$ 0.036	6.13a $\pm$ 0.002	3.85b $\pm$ 0.005	9.66a $\pm$ 0.028	163.05a $\pm$ 0.408	16.86a $\pm$ 0.014	2.85a $\pm$ 0.015	0.00	19.71a $\pm$ 0.020	182.76a $\pm$ 0.610

Values are expressed as mean $\pm$ SDThe values in the same columns denoted with different letters differ significantly at $p \leq 0.05$

reported, GLS in Brussels sprouts are less stable in the vegetable than leached into cooking water.

Looking at the changes in GLS content under the influence of sous-vide and traditional cooking, compared to the initial value (that was GLS in steamed vegetable taken), it was shown that sous-vide cooking, of both cauliflower and Brussels sprouts, led to higher losses than cooking in water (Fig. 1). The opposite tendency (both in indole and aliphatic GLS) was observed only in the case of broccoli. The possible reason could be different water activity, being one of the factors influencing myrosinase activity. The differences among species of *Brassica* vegetables in susceptibility for thermal and enzymatic degradation of GLS could be another important issue. The obtained results indicate that cooking at a lower temperature after previous vacuum packaging can be advantageous form of broccoli processing, among studied vegetables. Given the results concerned on GLS content in stored vegetables, however, it should be noted that losses, in comparison with values gained immediately after the process, were the highest (among all vegetables) in sous-vide cooked broccoli and reached over 30% (Tables 3, 4). Meanwhile, the losses observed in other vegetables were between 4 and 20%. From these results, it is considered that the use of the sous-vide method, in the context of preserving as high a content of GLS as possible, is proper only in the case of broccoli destined for direct consumption (cook and serve system).

The storage at 2 ± 1 °C of sous-vide-cooked broccoli caused a significant decrease of the glucoraphanin content (Table 4). After 5 days of storage, steamed cauliflower contained markedly less gluconapin. Meanwhile, in the case of Romanesco-type cauliflower and Brussels sprouts, storage was particularly disadvantageous for vegetables prepared

by the sous-vide method. They were characterized by significantly less amount of progoitrin, gluconasturcin (Romanesco-type cauliflower), and also gluconapin (Brussels sprouts). 5-day storage of broccoli and Brussels sprouts subjected to traditional cooking led to the losses, mainly in aliphatic GLS, whereas cauliflowers—indole GLS.

Further studies are needed to assess not only the effect of sous-vide method on the level of GLS, but also their breakdown products. It is important because of the anticancer properties of these compounds.

Conclusions

Cooking of vegetables by the sous-vide method is used as the alternative to traditional techniques of dish preparation. We found that the losses of dry matter (having regard to process efficiency) were slight in the case of vegetables cooked by the sous-vide method, whereas the traditional cooking of all the examined vegetables led to a statistically significant decrease in the dry matter content as compared to the raw material.

The usage of a lower temperature during cooking by the sous-vide method did not affect the quality and microbiological safety of the vegetables.

In the examined vegetables, nine GLS were identified. According to literature data, the estimation of GLS content in raw vegetables is quite difficult (underestimated). Our results showed that a higher (in comparison to raw vegetable) level of GLS was found in steamed vegetables. Thus, the content of these compounds in steamed vegetables was taken as an initial amount of GLS.

Fig. 1 Percentage changes in GLS content in vegetables subjected to a sous vide or traditional cooking, as compared to the initial value (GLS content in steamed vegetables regarded as 100%)

Glucoraphanin, glucoiberin, progoitrin and gluconapin, glucobrassicin as well as 4-metoxylucobrassicin were in higher amounts in broccoli prepared by the sous-vide method, compared to the vegetables cooked traditionally in water.

Cooking of Brussels sprouts and Romanesco-type cauliflower by the sous-vide method resulted in much higher losses of GLS, in comparison with the traditional cooking. It is to emphasize that the use of this technique of processing was particularly disadvantageous and led to a decrease in the total content of GLS, also as compared to the raw vegetable.

Our results may indicate that SV cooking can be an advantageous processing method of broccoli destined for direct consumption.

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Conflict of interest The authors declare that they have no conflict of interest.

Compliance with ethics requirements This article does not contain any studies with human or animal subjects.

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