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EFFECT OF CONVENTIONAL COOKING ON CHANGES IN THE CONTENTS OF BASIC COMPOSITION AND GLUCOSINOLATES IN KALE

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Abstract: *Brassica* vegetables have been strongly recommended as part of human diet because of its high content of bioactive sulphur compounds, *eg* glucosinolates. The nutrient and health-promoting compounds in kale are significantly affected by traditional cooking. The study investigated changes in the levels of dry mass, ash, fat, total protein, dietary fibre as well as total and individual glucosinolates in the kale due to the traditional cooking process. As a result of cooking kale, a significant decrease was noted in the content of fat, dry matter, indole glucosinolates, and a significant growth in the content of protein, ash, dietary fibre, and aliphatic glucosinolates compared to the raw vegetable.

Keywords: kale, glucosinolates, traditional cooking, dietary fibre, basic composition

Introduction

The *Brassicaceae* family comprises about 400 genera and 4000 species of vegetables, which are commonly grown and popular everywhere in the world. They are abundant in health-promoting phytochemicals [1]. The kale (*Brassica oleracea* L. var. *acephala*), being a traditional, biannual leafy vegetable of this family, is grown worldwide. Of the *Brassica* vegetables, kale was found to have the highest antioxidant activity and large concentrations of vitamins, minerals, dietary fibre, glucosinolates, chlorophyll-associated carotenoids, flavonoids, and phenolic acids [2].

According to the epidemiological studies, there is an opposite relationship between consumption of *Brassica* vegetables and occurrence of certain cancer forms, cardio-

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vascular and degenerative diseases, immune disfunction and aged-related macular degeneration [3, 4]. Plant-derived bioactive compounds can be classified, according to their chemical structure, as antioxidants, vitamins, polyphenols, terpene derivatives, sulphur compounds, phytoestrogens, minerals, polyunsaturated fatty acids, dietary fibre, and phytic acid. The sulphur compounds are commonly found in *Brassica* or allium vegetables [5].

The glucosinolates (GLS) belong to a large group of sulfur-containing compounds occurring in all the *Brassicas* vegetables of economic importance. Usually, their structure includes: the β-D-thioglucose group, a sulfonated oxime moiety and a variable side-chain derived from methionine, tryptophan or phenylalanine, and some branched-chain amino acids. They are stored in the myrosinase-containing cells in the intact plant tissue. Upon tissue disruption, glucosinolates undergo hydrolysis by the endogenous enzyme 'myrosinase' (thioglucoside glucohydrolase EC 3 : 2 : 3 : 1) that in turn releases a range of breakdown products. Depending on the chemical structure of glucosinolates (aliphatic, aromatic, or indole glucosinolates), coexisting in vegetables factors like epithiospecifier proteins (ESP), Fe^{2+} or ascorbic acid, environmental conditions (pH value), the end products of hydrolyzed glucosinolates are different (isothiocyanates, nitriles, indoles, thiocyanates and oxazolidines) [6–10]. Furthermore, glucosinolates can also undergo thermal and chemical degradation. Some factors, like *eg*: ascorbic acid, $MgCl_2$, temperature, pH and pressure affect the activity of myrosinase. In addition, myrosinase-like activity is induced by the gastrointestinal microbiota. Ingestion of the glucosinolates-containing products in the absence of the active plant-derived myrosinase, still results in the formation and absorption of bioactive breakdown products obtained due to the gut microbiota enzymes. During digestion, such factors as the degree of cell disruption, time of gastrointestinal transit, individual genotype, meal composition, and diversity of the colonic microbiota can additionally affect glucosinolates [8, 11]. Until now, over 200 different naturally occurring glucosinolates have been identified in *Brassica* vegetables in relatively high amounts [3].

Some glucosinolates and their decomposition products have gained more widespread attention as chemopreventive agents. These are 4-methylsulphinylbutyl isothiocyanate (sulforaphane), indole-3-carbinol or 3,3'-diindolylmethane. Chemoprevention, according to its definition, is the use of natural or synthetic agents which have ability to reverse, inhibit, or prevent the development of a chronic-degenerative disease [12]. The advantageous effects of glucosinolates and their breakdown products have been thoroughly investigated. The studies revealed that glucosinolates and their breakdown products may to a certain degree regulate or adjust many essential processes like the inhibition of inflammatory processes; induction of the cytoprotective enzymes; modulation of the signaling pathways in cancer, including cellular proliferation, angiogenesis; the epithelial-mesenchymal transition; self renewal of cell in cancer stem; suppression of the diverse oncogenic signaling pathways; modulation of epigenetic alterations; and regulation of polycomb group proteins or epigenetic cofator modifiers [1, 3].

Glucosinolates along with the products of their decomposition (isothiocyanates, nitriles, thiocyanates, epithionitriles, and oxazolidines) exhibit also antioxidant, anti-inflammatory, anti-allergic, anti-fungal, anti-virus, anti-mutagenic, and anti-bacterial properties [13, 14]. As was reported by some researchers, not only breakdown products

of glucosinolates, but also intact glucosinolates could have a pro-health effect [1, 15]. The protection provided by phytochemicals occurring in the glucosinolates-containing kale is particularly important in the context of the safe and cost-effective strategy for combating several chronic diseases.

The glucosinolates content in *Brassica* vegetables prior to consumption vary markedly due to differences occurring at various stages of the food supply chain, including: cultivation; growth condition; climate; plant variety; the tissue-specific distribution in a plant parts (seeds, leaves, roots, stems); storage and packaging conditions; and culinary treatment [3, 16].

Generally, kale is not consumed immediately after harvesting. As storage and cooking affect pro-health components, heat treatment of food induces several biological, physical and chemical changes. When preparing food, cooking is the most commonly used technique in processing *Brassica* vegetables. The process has a great effect on the health-promoting bioactive compounds and elementary composition of these plants. Changes in phytochemicals due to cooking may result from two contrary phenomena: the heat-induced denaturation of enzymes which can catalyse the breakdown of nutrients and phytochemicals and an effect of matrix softening, due to which the extractability of phytochemicals increases, resulting in their higher concentration compared to the raw material. The results of previous studies concerning the effect of processing *Brassica* vegetables on glucosinolates content are sometimes inconsistent or contradictory [8, 17]. Hence, it is necessary to evaluate the availability of the phytochemicals in human diet, discovering what happens to phytochemicals before and after food processing and monitor their final concentration.

The aim of processing is therefore to enhance beneficial properties of *Brassica* vegetables by improving bioavailability of glucosinolates and other compounds as well as extending shelf life. The quality of cooked vegetables depends on quality of the raw material, parameters and methods of processing, the variety of *Brassica* vegetables, and the kind of compounds [2].

The objective of this work was also to examine to what extent the commonly used process of thermal processing of kale changes the selected parameters of its health quality such as dry matter content, total protein, fat, ash, dietary fibre, and individual and total glucosinolates, which are important and potentially health-promoting constituents of kale. In order to achieve this aim, we hypothesised that the dry matter, total protein, fat, ash, dietary fibre, as well as individual and total glucosinolates contents in the raw and cooked kale differed statistically significantly. In general, this study was undertaken to broaden knowledge on the health-promoting properties of the raw and conventionally cooked kale in terms of the above indicators.

Material and methods

Plant material

The material investigated consisted of fresh kale (*B. oleracea* L. var *acephala*) leaves and leaves after cooking to consumption consistency. The kale cultivar under investiga-

tion was *Winterbor* F_1 and was grown up at the Polan Plant and Horticultural Seed Production Centre in Krakow, Poland (Experimental Station in Igolomia). The experimental field was located in the eastern outskirts of the Krakow. The kale was grown in black soil on loess framework with neutral pH. Mineral fertilization was applied according to the fertility of soil and the nutritional requirements of the species and condition treatments were carried out during the growing season (depending on soil and weather conditions).

Vegetable samples were prepared for analyses directly after harvest. The leaves were firstly separated (5 kg green mass), then washed under running water, next tiny cut into strips 2–3 cm in width (exterior and interior parts of the plant) and mixed in order to obtain the representative average laboratory samples (a minimum of three for each analysis performed on the fresh material and the same procedure was done on material after cooking). Another part of fresh material was washed and then dried on the filter paper; shredded mechanically; frozen at –22^{o}C; and next freeze-dried in the Christ Alpha 1–4 apparatus (Christ, Germany). The material, having undergone freeze-drying, was additionally comminuted in the Knifetec 1095 Sample Mill (Tecator, Sweden) until reaching a homogenous sample with possibly the smallest particle diameter. At the same time, the other vegetable batch was cooked in the traditional way (by domestic cooking methods), in a stainless steel pot on the electric stove top. Vegetables were cooked in unsalted water and in the initial phase of hydrothermal treatment – without a lid but in accordance with the principle "from farm to fork". The proportion of water to the raw material being 5 : 1 by weight. The cooking time applied was 15 minutes. The boiled vegetables were then prepared as described for fresh vegetables.

Analytical methods

The dry matter of the prepared samples of vegetables was determined according to PN-90/A-75101/03 [18]. The determination principle comprised determining the decrease in mass upon removal of water from the product during thermal drying at the temperature of 105^{o}C, under normal pressure conditions.

In raw freshly and lyophilized kale the following analyses were also performed: protein content using Tecator Kjeltec 2200 (Tecator, Sweden); fat content, using Tecator Soxtec Avanti 2050 (Tecator, Sweden); ash content by dry mineralisation in mufl Snol 8.2/1100 (Snol, Lithuania) on oven at 525^{o}C (PN-A-79011-8:1998 [19]) and dietary fibre content with enzymatic-gravimetry method using Tecator Fibertec System E (Tecator, Sweden).

The procedure of protein determination employ mineralization of the product in concentrated sulphuric acid (IV) ("the aqueous mineralization"), followed by alkalizing the solution, distillation of ammonia released and its qualitative determination according to PN-EN ISO 8968-1:2004 [20].

Fat determination is based on the extraction of fat from the dried material with an organic solvent (petroleum ether), distilling off the solvent, drying the residue and determining the weight of the extracted "crude fat" according to PN-A-79011-4:1998 [21].

Dietary fibre content was determined according to PN-A-79011-15:1998 [22] by means of enzymatic and gravimetric methods. Lyophilized samples of kale were subjec-

ted to gelatinization with a thermally stable α-amylase, then digested by enzymes involving protease and amyloglucosidase to remove protein and starch present in the sample. Soluble dietary fibre was precipitated by adding ethanol. The sediment was then filtered off, washed in ethanol and acetone and, after drying, weighed. Half the samples was analysed for the presence of protein and the remainder incinerated. Total dietary fibre has been calculated as the weight of sediment minus the weight of protein and ash.

Determination of glucosinolates

In order to determine the content of glucosinolates, the ISO 9167-1 method with modifications described by Kusznierewicz was used [23]. For this purpose, 200 mg of each lyophilized *Brassica* sample was extracted three times with boiling methanol (2 cm^3, 70%). The known amount of glucotropaeolin (0.2 cm^3, 5 mM, AppliChem GmbH, Darmstadt, Germany*)* was added to each sample just before the first extraction as an internal standard for the HPLC analysis. The extracted glucosinolates were purified on column filled with 0.5 cm^3 of DEAE Sephadex A-25 anion-exchange resin (Sigma Chemical Co., St. Louis, MO, USA). The column was washed with 2 cm^3 imidazole formate (6 M) and twice with 1 mL Millipore water and then loaded with 6 cm^3 of each extract. Afterwards sulphatase water solution (1.67 mg/cm^3, 250 mm^3) *(Helix pomatia* type H1, Sigma Chemical Co., St. Louis, MO, USA) was introduced onto the column and the columns were incubated for 12 h at room temperature. Next day, the desulfo-glucosinolates were eluted with deionized water (2 · 0.75 cm^3) and injected (50 mm^3) into LC-DAD-ESI-MS system (Agilent Technologies, Wilmington, DE, USA) using a Grace Altima HP AQ RP-C18 column (150 · 4.6 mm, 3 mm). The mobile phase contained water (A) and acetonitrile/water (20 : 80, v/v, B). Chromatographic resolution was performed at 30°C with 1 cm^3/min flow rate and the following gradient program: linear gradient rinsing from 5% B to 100% B within 10 min and then isocratic separation with 100% B for 15 min. The chromatographic peaks were first detected by DAD (Agilent Technologies, 1200 series, Wilmington, DE, USA) at 229 nm, then the identity of individual ds-glucosinolates was confirmed by API-ESI-MS (Agilent Technologies, 6130 Quadrupole LC/MS, Wilmington, DE, USA). MS parameters were as follows: capillary voltage, 3000 V; fragmentor voltage, 120 V; drying gas temperature, 350°C; gas flow (N_2), 12 dm^3/min; nebulizer pressure, 35 psig. The instrument was operated both in positive and negative ion modes, scanning from m/z 100 to 800. The glucosinolates content of each sample was quantified by the internal standard method using glucotropaeolin – method according to ISO protocols (ISO Method 9167-1, 1992). However, in calculations of the content of individual glucosinolates, the updated UV response factors proposed by Clarke [24] were used. Glucosinolates concentrations are expressed in micromoles per gram of dry matter.

Statistical analysis

All analyses were carried out in three parallel replications and mean ± standard deviation (SD) were calculated for the values obtained (three independent pooled curly

kale samples were analysed). By the use of one-way analysis of variance (ANOVA), the significance of differences were checked between mean values of raw and cooked material. The significance of differences was estimated with the Duncan test at the critical significance level of $p \leq 0.05$. The Statistica 10.1 (StatSoft, Inc., USA) program was applied. The composition of glucosinolates was expressed as μmol/1 g dry matter.

Results

Basic composition and dietary fibre

As the dry matter content in the vegetable varies depending on the process applied, all the results presented below along with conclusions have been discussed basing on the results calculated per the dry matter unit. In consequence, only an effect of the process applied was shown.

The raw vegetable contained 17.20 g dry matter; 3.06 g proteins; 0.69 g fat; 0.51 g ash and 6.67 g dietary fibre/100 g fresh vegetable (Table 1).

Table 1

Basic composition of raw leaves of kale [g/100 g of fresh weight]

Component	Mean
Dry matter	17.20 ± 0.15
Total protein	3.06 ± 0.02
Fat	0.69 ± 0.01
Ash	0.51 ± 0.00
Total carbohydrates	13.00 ± 0.01
Dietary fiber	6.67 ± 0.00

Values are presented as mean value ± standard deviation (n = 3).

The process of cooking led to statistically significant ($p \leq 0.05$) reductions in the dry matter content and fat content, of 25.0 and 10.6%, respectively, compared to the raw vegetable (Table 2).

Table 2

Effect of cooking on basic composition in leaves of kale [g/100 g of dry matter]

Component	Raw	Cooked
Dry matter	17.20 ± 0.15^{a}	12.90 ± 0.04^{b}
Total protein	17.80 ± 0.11^{b}	20.20 ± 0.09^{a}
Fat	4.01 ± 0.03^{b}	3.59 ± 0.07^{a}
Ash	3.10 ± 0.00^{a}	3.90 ± 0.00^{b}
Total carbohydrates	75.20 ± 0.07^{b}	72.30 ± 0.05^{a}
Dietary fiber	38.80 ± 0.00^{b}	39.60 ± 0.00^{a}

Values are presented as mean value ± standard deviation (n = 3).
The values denoted with the same letters don't differ statistically significantly at $p \leq 0.05$.

Simultaneously, the same process resulted in statistically significant increases ($p \leq 0.05$) in the other components analysed like protein (13.4%), ash (25.8%) and dietary fibre (2.27%), compared to the vegetable before processing.

Total and individual glucosinolates

In the analysed kale, the following 6 aliphatic glucosinolates were identified: glucoiberin (GIB), progoitrin (PRO), sinigrin and glucoraphanin (SIN/GRA), gluconapin (GNA), glucoerucin (GER), as well as 3 indoles glucosinolates: glucobrassicin (GBS), metoxybrassicin (MGBs) and neo-glucobrassicin (neoGBS).

Total glucosinolate content in the raw kale was 2.25 μmol/g dry matter (Table 3). The content of aliphatic and indole glucosinolates in the total amount of these compounds was similar, being 49 and 51%, respectively. There was no the presence of aryl glucosinolates. The amounts of aliphatic and indole glucosinolates, expressed in absolute values, were respectively 1.10 and 1.15 μmol/g dry matter of raw material; sinigrin (SIN) and glucoraphanin (GRA) accounted for more than half of aliphatic glucosinolates (54.4%) and almost one-third of the total glucosinolates content (26.7%). Considering the absolute values, successive aliphatic glucosinolates occurring in raw kale were glucoiberin (GIB) (0.22 μmol/g dry matter) and glucoerucin (GER) (0.21 μmol/g dry matter). The proportion of aliphatic glucosinolates in total glucosinolates content was the lowest, for example, gluconapin (GNA) and progoitrin (PRO) occurred in kale in trace amounts (approx. 0.04 μmol/g dry matter).

Table 3

Content of glucosinolates in fresh and cooked kale [μmol/g of dry matter]

Glucosinolates	Kale raw	Kale cooked
Glucoiberin	0.22 ± 0.04^{a}	0.98 ± 0.04^{b}
Progoitrin	0.04 ± 0.02^{a}	0.20 ± 0.02^{b}
Sinigrin/Glucorafanin	0.60 ± 0.04^{a}	2.34 ± 0.02^{b}
Gluconapin	0.04 ± 0.00^{a}	0.10 ± 0.01^{b}
Glucoerucin	$0.21 \pm 0{,}02^{a}$	$0.17 \pm 0{,}02^{a}$
Glucobrassicin	0.53 ± 0.01^{b}	0.09 ± 0.02^{a}
Metoxyglucobrassicin	0.11 ± 0.02^{b}	0.02 ± 0.00^{a}
Neoglucobrassicin	0.50 ± 0.03^{b}	0.21 ± 0.04^{a}
Total gucosinolates	2.25 ± 0.09^{a}	4.21 ± 0.10^{b}

Values are presented as mean value ± SD (n = 3) and expressed in dry matter. Means in rows with different superscript letters in common differ significantly ($p \leq 0.05$)

Of the indole glucosinolates, the proportion of glucobrassicin (GBS) (46.5%) and neoglucobrassicin (neoGBS) (43.8%) in this group was the largest; the remainder being methoxyglucobrassicin (MGBS). In kale, glucobrassicin (GBS) content comprised 23.5% total glucosinolates amount (almost one-fourth), while neoglucobrassicin (neoGBS) 22.2%. On the other hand, after converting values to the vegetable dry matter, the

glucobrassicin (GBS) content in kale was 0.53 μmol/g, neoglucobrassicin (neoGBS) 0.50 μmol/g, and methoxyglucobrassicin (MGBS) 0.11 μmol/g.

As a result of cooking, there was a significant ($p \leq 0.05$) increase in the amount of total glucosinolates (of 87.4%) compared to the raw vegetable. In each of the analysed aliphatic glucosinolates increases were significant ($p \leq 0.05$); compared to the raw vegetables they were of 345.0% (GIB); 455.5% (PRO); 290.5% (SIN/GRA); 150.0% (GNA); and 40.0% (GER). Simultaneously, hydrothermal treatment resulted in a significant ($p \leq 0.05$) reduction in the level of indole glucosinolates: of 82.8% (GBS); 78.2% (MGBS); and 57.2% (neoGBS) compared to the raw vegetables.

Discussion

Basic composition

Dry mass

The content of dry matter in the raw kale was 17.2 g/100 g, which is similar to the results reported by other authors. According to the literature, dry mass content in raw kale fluctuates broadly from 10.4 to 21.19 g/100 g fresh vegetable [2, 25–27]. The dry matter content in the vegetable is affected by many factors, which may include variety as well as climatic conditions and agro-technical practices. This experiment revealed that during cooking dry matter content decreased by 25.0%. In the study of Florkiewicz et al [28] and Volden et al [14] cooking of fresh cauliflower and red cabbage caused significant decrease of dry matter level. A decrease in dry matter due to heat treatment in an aqueous environment may result from extraction of soluble components to water and/or absorption of the water by tissues as well as leaving some water on the surface of raw material, particularly if the surface is uneven and undulated [2]. On the other hand, in the paper of Gebczynski and Kmiecik [29] the increase of dry matter content was observed during boiling, probably because of the loss of water from the tissue and the contraction of the raw material.

Protein

The results obtained in this work are close to the values found by other authors. According to the literature data, protein content in raw kale ranges broadly from 2.4 to 9.6 g/100 g of the vegetable [27, 30, 31]. Protein in kale is regarded as the high grade protein due to large amounts of essential amino acids such as glutamic acid, aspartic acid, proline, and fewer levels of cysteine and methionine. The presence of above compounds and other exogenic aminoacids corresponds to great nutritive value of kale protein [27, 32]. As Almeida et al [33] reports, total protein content in kale is strongly affected by the conditions of cultivation (fertilization, kind and composition of soil) and variety. Deficiency of certain minerals in the soil, for example, phosphorous and potassium has an effect on the quantity and quality of protein in the vegetable [34].

This study revealed that cooking led to a 13.4% increase in protein content. Slupski et al [35] also reported that technological and culinary processing of New Zealand

spinach caused a significant increase in amino acid content in 100 g of edible portion, except for methionine and cystine in frozen products prepared for eating. On the other hand, Lisiewska et al [36] showed a 14% loss of protein during cooking Brussels sprouts, while the losses in protein content found by Czapski [37] and Florkiewicz et al [28] during cooking broccoli and cauliflower were of 15.6 and 10.5%, respectively. The increase in protein content in dry matter of the cooked kale could result from more efficient leaching of other constituents soluble in boiling water, which, in turn, increases the proportion of these constituents in 100 g dry matter.

Fat

As was reported by Ayaz et al [30], kale leaves contain mainly linoleic, α-linolenic and palmitic acids. In this work, the fat content in the examined raw kale was minimal (0.69 g per 100 g fresh weight of the vegetable) that almost fully agrees with the amounts found by Sikora and Bodziarczyk [27] and Skapski and Dabrowska [38], which were respectively 0.67 g and 0.4–1.3 g/100 g fresh vegetable. The process of cooking, investigated in this experiment, resulted in a 10.6% reduction of this component. Greater losses due to cooking, amounting to 57%, were reported by the US sources [39] and Florkiewicz et al [28] (25.3%), while Czapski [37] observed an increase in the fat content of 10.8%, when cooking broccoli.

Ash

The ash content determined in the raw kale in this work was 0.51 g/100 g vegetable fresh weight, which does not concur with the findings of other authors. Ash contents reported by other authors were within the range 1.1–2.18 g [27, 38, 40]. The process of cooking in this experiment, resulted in a 25.8% growth of this component, while Florkiewicz et al [28] reported the opposite trend. Among the green leafy vegetables, kale is an excellent source of minerals, especially accumulating high levels of calcium, phosphorus and magnesium. Calcium is easily assimilated, mainly due to the lack of oxalic acid, which limits its assimilation. Kale contains from 535 to 551 mg calcium, 117–106 mg magnesium [41], and 5.73 mg phosphorus per 1 g vegetable dry weight [30]. The most important microelements in kale are iron, zinc and manganese [30]. According Fadigas et al [41], 100 g of kale contains from 1.48 to 2.13 mg iron, 1.95–2.63 mg zinc and 1.34–2.05 mg manganese. Hence, due to these constituents this vegetable should be recommended, especially for children.

The apparent increase in ash content in dry matter of the cooked kale could result from more efficient leaching or by a potential release of cellular bound of other constituents soluble in boiling water, which increases the proportion of these constituents in 100 g dry matter.

Dietary fibre

The dietary fibre plays a significant role in the prevention of several diseases. *Brassica* vegetables are an excellent source of dietary fibre. In kale, the proportion of

non-digestible carbohydrates, *ie* dietary fibre comprising mainly water insoluble hemicelluloses and water soluble pectins responsible for the increased bacteria proliferation in the colon, is significant and ranges from 0.8 to 3.8% [31]. The content of dietary fibre in the examined raw kale was 6.67 g/100 g fresh vegetable weight, which agrees with the findings of Sikora and Bodziarczyk [27] (7.40–9.56 g/100 g). Thermal treatment of the examined vegetable, which was performed in this work, led to the small increase in dietary fibre content of 2.27%. Czapski [37] and Komolka and Gorecka [42] also observed an increase in dietary fibre content in other *Brassica* vegetables; the first in broccoli (26.9%) and the others in the white cabbage (56.7%), red cabbage (60.5%), and the Italian cabbage (38.5%). During wet heat processing, insoluble fibre can be broken into smaller fragments and then dissolved in the water.

In this study also the percentage of carbohydrates was calculated on as the difference between 100 g of fresh product and the sum of water (g), total fat (g), protein (g) and mineral compounds – ash (g). Fructose, glucose and sucrose are the major soluble sugars found in kale leaves [30]. It has been found in this work that 100 g the raw kale had 13.0 g total carbohydrates. The amounts reported by Sikora and Bodziarczyk [27] and American sources [39] were slightly lower and were respectively 10.14 g/100 g and 10.1 g/100 g. As Skapski and Dabrowska [38] demonstrated, in kale sucrose prevails over the simple sugars and the level of these constituents increases after the ground frost.

Cooking applied in this experiment caused a 3.94% decrease in the content of the aforementioned compounds. According to the USDA Nutrient Database for Standard Reference [39], the losses in carbohydrates due to cooking amounted to 35.6%; however, these noted by Czapski [37] in the cooked broccoli were much lower (10.8%).

Total and individual glucosinolates

The literature data on the total glucosinolates contents generally were close to the results obtained in this work [10, 43]. On the other hand, the value reported by Korus et al [16] much more exceeded the value obtained in the present work. According to the authors, usually there are two processes responsible for the reduction in the levels of glucosinolates: glucosinolates breakdown by myrosinase and thermal treatment of vegetables. During the first one, the enzyme initiates the process of hydrolysis that leads to an impairment of plant tissue and leakage of cell fluid [44]. The temperatures up to 60^{o}C enhance the activity of myrosinase, while higher temperatures lead to inactivation of the enzyme by denaturing the enzyme both in the cabbage and after leaking into the cooking water [45]. With regard to the second process, substantial glucosinolates losses due to this process were reported by Ciska and Kozlowska [46] and Volden et al [14]. Glucosinolates, as water-soluble, are leached out into the cooking water. Moreover, their heat resistance is various [47]. Most authors stated a decline in the amount of glucosinolates due to blanching and cooking in the selected *Brassica* vegetables, for example, in Brussels sprouts, white and green cauliflower, broccoli, and curly kale [48]; in kale [2]; in broccoli [49]; in cauliflower [14]; in broccoli [50]; in Brussels sprouts [51] or in cabbages [46, 52]. According to Kapusta-Duch et al [53] the process of

cooking *Brassica* vegetables caused a generally significant ($p \leq 0.05$) decrease in the total glucosinolates content compared with raw vegetables: 6.6% in the rutabaga; 68.9% in green cauliflower; and 69.2% in purple cauliflower. On the other hand, the authors found the increase in the levels of certain individual glucosinolates in rutabaga. As for the effects of different cooking techniques (boiling, steaming etc.) on glucosinolates content, these reported in the literature are not so clear-cut. There are however reports in the available literature, which indicate an increase in their amount resulting from cooking, which partially agrees with the results obtained in the present work. D'Antuono et al [54] reported that the total glucosinolates content was two-fold higher in boiled cauliflower in comparison to raw. The authors reported that this was due to higher extractability of these compounds in the cooked than in the raw plant tissues. According to Vallejo et al [55], inactivation of myrosinase and a breakdown of plant tissue upon heat provide partial explanation for their well-preserving or increase. Gliszczynska-Swigło et al [49] claimed that part of such molecules bound to the cell walls is released only after a breakdown of cell structures.

Ciska et al [51] found that 5-minute cooking Brussels sprouts led to a significant increase in the majority of aliphatic glucosinolates and one indole glucosinolates compared to the raw vegetable. Such trend was observed for glucoiberin, progoitrin, sinigrin, gluconapin, glucoraphanin, and glucobrassicin. However, these increases were not as high as in this work. After 15-minute cooking Brussels sprouts, an increase was significant only in case of glucoiberin and sinigrin. Extended cooking time (30 minutes) caused losses in the number of all individual glucosinolates. Ciska et al [51], Oerlemans et al [56] and Rosa and Heaney [57] noted that losses due to cooking were greatest in the case of indole glucosinolates, which is consistent with our findings. According to Vallejo et al [55], cooked broccoli contained significantly more glucoiberin, glucoalyssin, and progoitrin. Verkerk and Dekker (2004) and Oerlemans et al [56] observed an increase in total glucosinolates in steamed and conventional boiled red cabbage of 60% and 35%, respectively, compared to the raw vegetable, while the increase of total glucosinolates level determined by Roque-Sala [59] in Brussels sprouts was of 86% when compared to those before steaming (steaming time – approx. 5 minutes). Ciska et al [51] found that sinigrin and glucoraphanin showed the highest thermal stability during cooking. On the other hand, Sosinska and Obiedzinski [60] noticed that there are various factors affecting these differences, such as species, variety, storage conditions, cooking time, and the degree of sample fragmentation. As was also reported by Miglio et al [61], broccoli cooked by steaming had higher glucosinolates level compared to the fresh ones but the level was also higher when compared to those cooked by a conventional method. Numerous authors report that myrosinase is inactivated, for example, after: 5 minutes [62]; 2–5 minutes, depending on the applied treatment techniques [63]; or 4.8 minutes [58]. Researchers explain the phenomenon of an increase in these compounds by extractiveness of glucosinolates from the vegetable material after heating that in turn gives higher amounts of the glucosinolates accessible to extraction and determination [60]. In order to reach maximum activity, myrosinase, like other enzymes, must have appropriate conditions (pH, temperature, presence of co-factors such as ions of iron and vitamin C or the presence of other proteins) [64].

The differences observed in glucosinolates thermal degradation may be caused by the specific plant components negatively influencing glucosinolates stability. The higher glucosinolates content in plant tissue, the higher is their sensitivity to heat degradation. The process of cooking causes that some components, like glucosinolates, migrate into water and become diluted. Probably, there may be another mechanism affecting the variations in the glucosinolates thermal stability in different environments.

The results obtained indicate that further studies are needed to explain changes in glucosinolates contents during convectional cooking not only kale, but other *Brassica* vegetables. As the results presented by other authors and those obtained in this work are not clear-cut, the research problem seems to be interesting. Therefore, the aim should be to optimize hydrothermal processes in order to make the best possible use of pro-health substances occurring in *Brassica* vegetables in human nutrition.

Conclusions

The current study clearly shows that nutrient and health-promoting compounds in kale are significantly affected by traditional cooking. Raw kale (var. *Winterbor* F_1) was characterized by the rich primary composition and the significant content of glucosinolates.

The commonly used thermal processing method resulted in a statistically significant ($p \leq 0.05$) increase in the level of total protein, ash, dietary fibre, total and aliphatic glucosinolates and a substantial reduction in the content of fat, carbohydrates, total dry matter, indole glucosinolates compared to the raw vegetable.

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WPŁYW GOTOWANIA NA ZMIANY ZAWARTOŚCI SKŁADU PODSTAWOWEGO I GLUKOZYNOLANÓW W JARMUŻU

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Abstrakt: Warzywa kapustne są szczególnie zalecane w diecie ze względu na wysoką zawartość składników biologicznie czynnych zawierających siarkę, tj. glukozynolanów. Celem pracy było sprawdzenie jak zmieniają się wybrane parametry jakości zdrowotnej jarmużu (tj. zawartość: białka, tłuszczu, popiołu, węglowodanów, błonnika pokarmowego, suchej masy i glukozynolanów) pod wpływem tradycyjnego gotowania w wodzie. W wyniku omawianego procesu zaobserwowano istotne statystycznie obniżenie zawartości suchej masy, tłuszczu oraz glukozynolanów indolowych, a także istotny statystycznie wzrost zawartości białka, popiołu, błonnika pokarmowego oraz glukozynolanów alifatycznych, w stosunku do surowego warzywa.

Słowa kluczowe: jarmuż, glukozynolany, proces gotowania, błonnik pokarmowy, skład podstawowy