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Effect of cooking on the contents of glucosinolates and their degradation products in selected *Brassica* vegetables

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A B S T R A C T

Changes in the levels of glucosinolates and their degradation products in selected *Brassica* vegetables due to the cooking process were investigated. The purple cauliflower was found to be the best source of aliphatic and indole glucosinolates, and it was also abundant in compounds such as sinigrin, glucoraphanin and glucobrassicin. As a result of cooking ru-tabaga, green cauliflower and purple cauliflower, a significant decrease was noted in total glucosinolates (6.6, 68.9 and 69.2%, respectively) compared to raw vegetables. The hydro-thermal processing applied led to a decline in the sum of indoles and isothiocyanates of 48.5 and 11.0%, respectively, in green cauliflower; and of 75.8 and 42.4%, respectively, in purple cauliflower; whereas, in rutabaga it led to an increase of 142.9 and 329.4%, respectively, com-pared with raw vegetables.

Keywords: Brassica, vegetables Functional, food Glucosinolates, Breakdown products of glucosinolates, Traditional cooking

1. Introduction

Nowadays, there is increasing interest in natural, safe and functional foods. A food is said to be 'functional' if it provides a preventive, protective and/or curative function against one or more diseases, in addition to its adequate nutritional benefits (Kazeem & Davies, 2016).

Over the last two decades crops from the *Brassicaceae* (formerly Cruciferae) have been the focus of intense research based on their human health benefits (Traka & Mithen, 2009). Numerous epidemiological and pharmacological studies have revealed that a diet rich in *Brassica* vegetables may play an important role in the protection against many chronic diseases, including cardiovascular disease, type II diabetes, dementia, age-related macular degeneration, immune dysfunction, obesity and some cancers (Dos Reis et al., 2015; Shahidi & Ambigaipalan, 2015). The *Brassicaceae* is a plant family of huge economic importance, containing about 340 genera and 3700 species (Pedras & Yaya, 2010). These vegetables are among the most important vegetables consumed in Europe and all over the world owing to their availability at local markets throughout

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the whole year, affordable cost and consumer preference (Herr & Büchler, 2010; Hounsome, Hounsome, Tomos, & Edwards-Jones, 2009). Although horticultural *Brassicaceae* plants are excellent sources of nutrients, such as vitamins, minerals and fibre, the majority of the research has concentrated on the content of secondary metabolites, e.g. glucosinolates, polyphenols and others (Kapusta-Duch, Kopeć, Piątkowska, Borczak, & Leszczyńska, 2012; Manchali, Murthy, & Patil, 2012). An extensive body of literature strongly suggests that all these compounds exert physiological actions contributing to beneficial effects on human nutrition. Therefore, *Brassica* vegetables are classified as functional foods.

The glucosinolates (GLS) are a large group of sulphur-containing compounds that occur in all economically important varieties of *Brassica* vegetables. Their common structure comprises a β-D-thioglucose group, a sulphonated oxime moiety and a variable side-chain derived from methionine, tryptophan or phenylalanine and some branched-chain amino acids. The glucosinolates are themselves not biologically active, only their enzymatic derivatives are. When the plant tissue is damaged, the glucosinolates are hydrolysed by the endogenous enzyme 'myrosinase' (thioglucoside glucohydrolase EC 3:2:3:1) to release a range of breakdown products. Depending on the chemical structure of glucosinolates such as aliphatic, aromatic, or indole glucosinolates, coexisting factors in vegetables such as epithiospecifier proteins (ESP), ascorbic acid or Fe^{2+}, as well as environmental conditions such as pH value, and end products of hydrolysed glucosinolates are different, including isothiocyanates, indoles, nitriles, oxazolidines and thiocyanates (Girgin & El, 2015; Mithen, Dekker, Verkerk, Rabot, & Johnson, 2000; Tang, Paonessa, Zhang, Ambrosone, & McCann, 2013; Verkerk et al., 2009). Actually, more than 200 different naturally occurring glucosinolates that occur in relatively high levels have been identified in *Brassica* vegetables. The content of glucosinolates depends on many factors, such as plant variety, growing condition, climate, the tissue-specific distribution in a plant parts (seeds, stems, leaves and roots), and both storage conditions (type and duration) and culinary treatment (Fuentes, Paredes-Gonzalez, & Kong, 2015; Korus, Słupski, Gębczyński, & Banaś, 2014).

The protection provided by phytochemicals present in *Brassica* vegetables containing glucosinolates is of great interest, because they may provide a safe and cost-effective strategy for combating several chronic diseases. However, according to some scientists, not only breakdown products of glucosinolates, but also intact glucosinolates are responsible for their health-promoting effect (Abdull Razis, Bagatta, De Nicola, Iori, & Ioannides, 2010; Avato & Argentieri, 2015). Bioactivity or biological effects due to intact glucosinolates present in food or in forage of monogastric animals result from the glucosinolates and/or transformation products, which in turn occur in non-enzymatic and/or myrosinase-catalysed processes (Avato & Argentieri, 2015). In *Brassica* vegetables, glucoraphanin and glucobrassicin are the most desirable glucosinolates, while indole-3-carbinol is one of the most valuable glucosinolate breakdown products. Its chemopreventive action, as in the case of sulforaphane, is widely known and documented. It is responsible for, among others, the induction of phase I and II detoxification enzymes (Higdon, Delage, Williams, & Dashwood, 2007; Kim & Milner, 2005; Murray, 2006), the inhibition of DNA adducts as well as inhibition of cell proliferation (Nachshon-Kedmi, Yannai, Haj, & Fares, 2003), cell cycle arrest (Moiseeva, Heukers, & Manson, 2007; Pappa et al., 2006), the inhibition of invasive growth and angiogenesis (Wu, Lin, & Chen, 2005), the induction of apoptosis (Souli, Machluf, Morgenstern, Sabo, & Yannai, 2008), and anti-estrogenic activity (Rahman, Li, Wang, Sarkar, & Sarkar, 2006). The compound 3,3′-diindolylmethane shows similar activity to indole-3-carbinol, and in addition exhibits the ability to repair DNA (Kim & Milner, 2005). Isothiocyanates, including the one most widely known in the literature, i.e. 4-methylsulphinylbutyl isothiocyanate (sulforaphane), have a similar protective effect against cancer as the aforementioned indole compounds. The isothiocyanates, however, inhibit the activity of certain enzymes involved in the activation of xenobiotics (phase I) and they induce phase II enzymes, thereby reducing the amount of any active carcinogen (Śmiechowska, Bartoszek, & Namieśnik, 2008). Sulforaphane also has anti-inflammatory and antimicrobial activities against *Helicobacter pylori* (Fimognari & Hrelia, 2007). In contrast to isothiocyanates, the indole compounds induce enzymes of both phases I and II of detoxification (Nho & Jeffery, 2001). Knowledge about the bioavailability, transport and metabolism of glucosinolates after consumption of *Brassica* vegetables is an essential prerequisite to understand the mechanisms of their protective effects observed in humans (Moreno, Carvajal, López-Berenguer, & García-Viguera, 2006). The occurrence of such compounds in food determines its functional and health-promoting nature (Manchali et al., 2012; Volden, Bengtsson, & Wicklund, 2009). Therefore, their consumption should be highly promoted.

In most cases *Brassicas* are not eaten immediately after being harvested; hence, storage and cooking impact upon health beneficial components. Processing of *Brassica* vegetables has complex influences on the food matrix affecting the level of glucosinolates: enzymatic hydrolysis by myrosinase, myrosinase inactivation, cell lysis and leaching of glucosinolates, their breakdown products and myrosinase in cooking water, thermal degradation of glucosinolates and their breakdown products, increase in the chemical extractability, and loss of enzymatic cofactors (Verkerk et al., 2009). The objectives of this paper were to evaluate how the process of traditional cooking in water changes the content of total and individual glucosinolates and their breakdown products (indole-3-carbinol, indole-3-acetonitrile, 3,3′-diindolylmethane, total indoles and total isothiocyanates) in common *Brassica* vegetables (green and purple cauliflower, rutabaga).

The concentration of glucosinolates and isothiocyanates in *Brassica*-vegetable-based food is far more variable than previously thought. Interestingly, in recent years, many studies have reported different influences of cooking on the chemical compositions of vegetables (Palermo, Pellegrini, & Fogliano, 2014). In this study, analysis was carried out not only for changes in total glucosinolates, but also in individual glucosinolates and, innovatively, their breakdown products, influenced by the cooking process. To the best of our knowledge there are, unfortunately, very few studies that could investigate not only the amount of glucosinolates and their profile, but also their breakdown products. The majority of studies published to date have focused on other cultivars of *Brassicas*. Less information is available about other *Brassicas*, e.g. coloured varieties of

cauliflower (purple and green) or rutabaga. The thermostability of glucosinolates varied not only in different media such as boiled water, but also the environment in cells or cell compounds of the various cultivars and plant parts (e.g. cauliflower versus rutabaga) can exert a significant influence on the thermal stability of glucosinolates. In general, this study was undertaken to broaden knowledge on the health-promoting properties of raw and conventionally boiled (the most common and popular thermal processing) cauliflower (green and purple) and rutabaga, particularly in terms of the following indicators: total and individual glucosinolates, indole-3-carbinol, indole-3-acetonitrile, 3,3′-diindolylmethane, total indoles and total isothiocyanates.

2. Materials and methods

2.1. *Plant material*

The material investigated consisted of three species of *Brassicas* that are cultivated and eaten in Poland: green cauliflower – *Brassica oleracea L. var. botrytis L.* (Vita Verde cv.), and purple cauliflower – *Brassica oleracea L. var. botrytis L.* (Graffiti cv.); and rutabaga – *Brassica napus L. var. napobrassica* (Wilhelmburska cv.). The vegetables were cultivated at the "Polan" Plant and Horticultural Seed Production Centre Ltd. Krakow (Poland) and the "Traf" Producers' Cooperative in Tropiszów (Poland).

Vegetable samples were prepared for analyses directly after harvest. From a 5 kg batch of every vegetable, samples were taken from the usable parts, which were then cut along the axis in a ratio of 1:4 or 1:8 (depending on size), washed under running water, dried on filter paper, shredded mechanically, frozen at –22 °C, and then freeze-dried in a Christ Alpha 1–4 apparatus (Christ, Germany). Having undergone freeze-drying, the material was additionally comminuted in a Knifetec 1095 Sample Mill (Tecator, Sweden) until a homogenous sample with the smallest possible particle diameter was produced. At the same time, another vegetable batch was cooked in the traditional way (by domestic cooking methods), in a stainless steel pot on an electric hob. 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 was 5:1 by weight. The cooking time applied was 15 min. The boiled vegetables were then prepared as described for fresh vegetables.

2.2. *Analytical methods*

2.2.1. *Determination of glucosinolates*

In order to determine the content of GLs, the ISO 9167-1 method with modifications described by Kusznierewicz was used (Kusznierewicz, Iori, Piekarska, Namiesnik, & Bartoszek, 2013). For this purpose, 200 mg of each lyophilized *Brassica* sample was extracted three times with boiling methanol (2 mL, 70%). A known amount of glucotropaeolin (0.2 mL, 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 GLs were purified on a column filled with 0.5 mL of DEAE Sephadex A-25 anion-exchange resin (Sigma Chemical Co., St. Louis, MO, USA). The column was washed with 2 mL imidazole formate (6 M) and twice with 1 mL Millipore water and then loaded with 6 mL of each extract. Afterwards, sulphatase water solution (1.67 mg/mL, 250 μL) *(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. On the next day, the desulfo–GLs were eluted with deionized water (2 × 0.75 mL) and injected (50 μL) into an LC-DAD-ESI-MS system (Agilent Technologies, Wilmington, DE, USA) using a Grace Alltima 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 mL/min flow rate and the following gradient programme: linear gradient rinsing from 5 to 100% B within 10 min and then isocratic separation with 100% B for 15 min. The chromatographic peaks were first detected via DAD (Agilent Technologies, 1200 series, Wilmington, DE, USA) at 229 nm, and then the identity of individual ds-GLs was confirmed via 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 L/min; nebulizer pressure, 35 psig. The instrument was operated both in positive and negative ion modes, scanning from m/z 100 to 800. The GL level of each sample was quantified with the internal standard method using the glucotropaeolin method according to ISO protocol (ISO:9167-1, 1992). However, in calculations of the content of individual GLs, the updated UV response factors proposed by Clarke (2010) were used. GLs concentrations are expressed in micromoles per gram of dry weight.

2.2.2. *Determination of isothiocyanate*

The content of ITC was determined according to Zhang, Wade, Prestera, and Talalay (1996) with some modifications described by Piekarska et al. (2014). Lyophilized *Brassica* samples (0.2 g) were mixed with 5 mL of 0.01 M sodium phosphate buffer (pH 7.4) and incubated for 3 h at 37 °C. The samples were centrifuged (3005 × g, 10 min, 4 °C, Thermo Scientific Heraeus Megafuge, Karlsruhe, Germany) and the collected supernatants were passed through a Bakerbond SPE C18 500 mg cartridge (JT Baker, Phillipsburg, NJ, USA) preconditioned with 6 mL of methanol and 6 mL of 10% methanol in water (v/v). The retained ITC (together with indolic breakdown products) were then eluted with 1 mL of methanol. The concentrated ITC (0.1 mL of the eluate) was submitted to a cyclocondensation reaction in a mixture containing 0.5 mL of 2-propanol, 0.5 mL of 0.1 M potassium phosphate buffer (pH 8.5), and 0.1 mL of 60 mM 1,2-benzenedithiol dissolved in 2-propanol. The mixture was incubated for 1 h at 65 °C. The product of the reaction, 1,3-benzenedithiol-2-thione, was analysed via reverse phase HPLC coupled with a DAD detector (Agilent Technologies, 1200 series, Wilmington, DE, USA). The reaction mixture (30 μL) was injected onto a 150 mm × 4.6 mm, 3.5 mm Zorbax Eclipse XDBC8 column. The mobile phase used was 1% (v/v) formic acid in water (A) and methanol (B) with a flow rate of 1 mL/min. The gradient changed as follows: 60 to 100% B within 12 min, then 100% B for 3 min. Chromatograms were traced at 365 nm. For quantification of the reaction product, a standard line was constructed using a series of 1,3-benzenedithiol-2-thione solutions with a concentration range of 0.01–10 mM.

2.2.3. *Determination of indolic compounds*

The indolic compounds were analysed with a method described earlier (Piekarska et al., 2014; Pilipczuk, Kusznierewicz, Namieśnik, & Bartoszek, 2015). The eluates from SPE obtained as described for ITC were also used to determine the indole content in the *Brassica* samples. An HPLC-DAD-FLD system (Agilent Technologies, 1200 series, Wilmington, DE, USA) equipped with a Zorbax Eclipse XDB-C8 column was used for the analysis of indolic compounds. A linear gradient of acetonitrile in water ranging from 10 to 100% within 30 min and a mobile phase flow rate set at 1 mL/min ensured the satisfactory resolution of indoles. The injection volume of the extract was 20 µL. Fluorescence emission at 360 nm (excitation at 280 nm) enabled monitoring of indolic analytes. The calibration curves used for their quantification were generated by the integration of the areas of fluorescence peaks determined during analysis of serial dilutions of I3C (indole-3-carbinol, Sigma Chemical Co., St. Louis, MO, USA), I3ACN (indole-3-acetonitrile, Merck, Darmstadt, Germany), I3AA (indole-3-acetic acid, Merck, Darmstadt, Germany), and DIM (3,3′-diindolylmethane, Sigma Chemical Co., St. Louis, MO, USA).

2.3. *Statistical analysis*

All analyses were carried out in three parallel replications and mean ± SDs were calculated for the values obtained. One-way analysis of variance (ANOVA) was used to check the significance of differences 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) programme was applied. The composition of GLS was expressed as µmol/1 g dry matter.

3. Results and discussion

3.1. *Glucosinolates in raw vegetables*

In this study, all vegetables were grown under similar environmental conditions, and as the vegetables were processed on the day of harvest, the post-harvest changes are considered to be minimal. Analyses of the levels of glucosinolates were carried out in three replications in fresh and cooked material. In the analysed *Brassica*, i.e. green cauliflower, purple cauliflower and rutabaga, the aliphatic glucosinolates identified were: glucoiberin (GIB), progoitrin (PRO), sinigrin and glucoraphanin (SIN/GRA), gluconapoleiferin (GNF), gluconapin (GNA), glucobrassicanapin (*GBN*), glucotropaeolin (*GTL*), glucoiberverin (GIV), *glucoerucin* (*GER*), and glucoalyssin (*GAL*); as well as indoles: glucobrassicin (GBS), *metoxybrassicin* (MGBs) and neo-glucobrassicin (neoGBS); along with one aryl: *gluconasturcin* (GNT); and one unknown (in rutabaga), labelled x, with a molecular weight of 435 Da (Table 1).

The compounds from the glucosinolates group demonstrate high chemical stability; the occurrence of biologically active indoles and isothiocyanates results from the myrosinase-catalysed enzymatic hydrolysis of glucosinolates. The enzyme (alternative names: β-thioglucosidase, thioglucoside glucohydrolase, EC 3.2.3.1.) is present in the myosin cells, from which it is released under appropriate conditions. In the case of *Brassica* vegetables, the glucosinolate decomposition leads to the formation of both stable (allyl isothiocyanates and *2-fenyloetyl*) and unstable (e.g. indole-3-carbinol) products (Grubb & Abel, 2006). Differences in chemical properties and biological activity between glucosinolates and their respective hydrolysis products are largely determined by the side-chain structure (Mithen et al., 2000). It has been proved that when plant-derived myrosinase is in the diet, glucosinolates are

Table 1 – Content of glucosinolates in fresh and cooked green cauliflower, purple cauliflower and rutabaga (µmol/g d.w.).

Glucosinolates	Green cauliflower fresh	Green cauliflower cooked	Purple cauliflower fresh	Purple cauliflower cooked	Rutabaga fresh	Rutabaga cooked
Glucoiberin	0.337 ± 0.03^{b}	0.143 ± 0.04^{a}	1.915 ± 0.02^{c}	0.909 ± 0.02^{d}	–	–
Progoitrin	0.399 ± 0.08^{d}	0.135 ± 0.01^{b}	0.269 ± 0.01^{c}	0.064 ± 0.01^{a}	2.624 ± 0.02^{e}	2.772 ± 0.04^{f}
Sinigrin/Glucorafanin	1.261 ± 0.03^{b}	0.494 ± 0.08^{c}	4.374 ± 0.07^{a}	1.244 ± 0.08^{b}	0.052 ± 0.02^{a}	0.063 ± 0.02^{b}
Gluconapin	0.167 ± 0.04^{c}	0.040 ± 0.01^{a}	0.085 ± 0.01^{b}	0.041 ± 0.01^{a}	0.453 ± 0.03^{d}	0.079 ± 0.00^{b}
Glucoiberviryn	1.813 ± 0.06^{e}	0.514 ± 0.10^{c}	0.683 ± 0.04^{d}	0.110 ± 0.05^{a}	0.370 ± 0.04^{b}	0.462 ± 0.01^{c}
Glucoerucin	0.796 ± 0.06^{e}	0.211 ± 0.04^{a}	–	–	0.486 ± 0.02^{d}	0.432 ± 0.01^{c}
Gluconastrucin	0.075 ± 0.02^{ab}	0.013 ± 0.00^{a}	0.077 ± 0.01^{b}	0.003 ± 0.00^{a}	0.867 ± 0.05^{d}	0.546 ± 0.01^{c}
Glucotropaeolin	–	–	0.158 ± 0.00^{b}	0.092 ± 0.01^{a}	–	–
Gluconapoleiferin	–	–	–	–	0.398 ± 0.02^{a}	0.553 ± 0.07^{b}
Glucoalisin	–	–	–	–	0.114 ± 0.21^{a}	0.210 ± 0.03^{b}
Glucobrassicanapin	–	–	–	–	0.456 ± 0.04^{b}	0.268 ± 0.01^{a}
X*	–	–	–	–	0.857 ± 0.04^{a}	0.965 ± 0.04^{b}
Glucobrassicin	0.249 ± 0.04^{c}	0.044 ± 0.02^{a}	0.298 ± 0.03^{d}	0.019 ± 0.00^{a}	0.020 ± 0.02^{a}	0.058 ± 0.02^{ab}
Metoxyglucobrassicin	0.045 ± 0.02^{a}	0.009 ± 0.00^{b}	0.092 ± 0.00^{c}	0.032 ± 0.01^{ab}	0.052 ± 0.01^{a}	0.101 ± 0.03^{c}
Neoglucobrassicin	0.120 ± 0.02^{c}	0.030 ± 0.01a	0.308 ± 0.03^{b}	0.030 ± 0.01^{a}	0.586 ± 0.06^{d}	0.347 ± 0.05^{b}
Total glucosinolates	5.265 ± 0.18^{c}	1.635 ± 0.15^{a}	8.260 ± 0.16^{f}	2.545 ± 0.07^{b}	7.340 ± 0.24^{e}	6.858 ± 0.06^{d}

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 ($p \leq 0.05$).

* Unknown glucosinolate, molecular weight of 435.

rapidly hydrolysed in the proximal gut. If myrosinase is inactivated, for example by cooking the vegetables prior to consumption, the ionized nature of glucosinolates may enable them to reach the distal gut where they could be metabolized by bacterial enzymes (Mithen et al., 2000). Myrosinase releases glucose and breakdown products. Glucosinolates are broken down by plant myrosinase in the small intestine or by bacterial myrosinase in the colon; their metabolites are detectable in human urine 2–3 h after consumption of *Brassica* vegetables. Interpretation of epidemiological data and the effective use of *Brassica* vegetables for human health requires an understanding of glucosinolates chemistry and metabolism, across the whole food chain, from production and processing to the consumer (Moreno et al., 2006). Therefore, since the representative vegetable samples had been prepared for freeze-drying and the vegetables underwent such preprocessing as cleaning, cutting, or grinding, the raw vegetables were also examined for glucosinolate breakdown products, namely indole-3-carbinol (I3C), indole-3-acetic acid (I3AA), indole-3-acetonitrile (I3ACN), 3,3′-diindolylmethane (DIM), total indoles and total isothiocyanate content (ITC).

3.1.1. *Total glucosinolates*

The total glucosinolates content in the individual vegetable types differed significantly ($p \leq 0.05$). Of the raw *Brassica* vegetables, the lowest mean content of total glucosinolates was found in green cauliflower (5.26 μmol/g dry matter), while the highest was in rutabaga (7.34 μmol/ g dry matter) and purple cauliflower (8.26 μmol/ g dry matter) (Table 1).

In terms of all the glucosinolates, the contribution of aryl glucosinolate was usually the lowest. They occurred in trace amounts in green and purple cauliflower (approx. 0.07 μmol/g dry matter) (Table 1). The exception was rutabaga, which contained fairly large quantities of *gluconasturcin* (0.87 μmol/g dry matter).

In general, the literature data confirm the results obtained in this study for total glucosinolates content. Sosińska and Obiedziński (2007) reported a similar content of total glucosinolates in green cauliflower (6.2 μmol/g dry matter), while the values registered by Volden et al. (2009) in green cauliflower (*var.* "*Emeraude*") and in purple cauliflower (*var.* "*Grafitti*") were much lower, i.e. 3.34 and 2.52 μmol/g dry matter, respectively. When examining rutabaga, Bradshaw (2010) identified a total of 12 glucosinolates, of which progoitrin (2-hydroxybut-3-enyl glucosinolate) comprised 42.9% of the mean total glucosinolate content. The goiter fraction, progoitrin, and gluconapoleiferin (2-hydroxypent-4-enyl glucosinolate), and the total glucosinolate content varied from 2.4 to 10.6 and 9 to 18.5 μmol/g d.m., respectively. These results are similar to our findings, according to which, of all the glucosinolates identified in rutabaga, progoitrin constituted 34.5% of total glucosinolates and half of aliphatic GLS.

Despite all these health benefits, recent experimental data suggest that *Brassica* vegetables, their glucosinolates and related breakdown products may also have genotoxic activity. It seems, however, unlikely that toxicity would be induced by consumption of *Brassica* vegetables due to the low amount of active compounds provided with a standard diet (Avato & Argentieri, 2015; Fimognari, Turrini, Ferruzzi, Lenzi, & Hrelia, 2012; Latté, Appel, & Lampen, 2011). In the past, glucosinolates and their breakdown products were mainly considered to be toxic and goitrogenic compounds. However, since most vegetables rich in 2-hydroxyalkenyl glucosinolates are cooked prior to consumption, goitrogenic effects should be of no concern in an average human diet (Hanschen, Lamy, Schreiner, & Rohn, 2014). Many organizations recommend the consumption of 5–9 servings (2½–4½ cups) of fruits and vegetables daily, but individual recommendations for *Brassica* vegetables have not been established (Higdon et al., 2007). The daily intake of glucosinolates is difficult to estimate due to the high diversity of constituents in various *Brassica* cultivars. Better understanding of the molecular mechanism responsible for the chemopreventive effects of these dietary compounds requires further studies on safety profile of dosage regimens as well as potential interactions between different glucosinolates and other diet constituents (Fuentes et al., 2015). In the United States isothiocyanates, but not their precursor glucosinolates, are classified as flavourings and listed as food additives. Their use as food additives is widespread in the USA, but so far only 3-methylthiopropyl-isothiocyanate and allyl isothiocyanate have been evaluated toxicologically and information has been available (Deng et al., 2015).

3.1.2. *Aliphatic and indole glucosinolates*

In terms of the total amount of these compounds aliphatic glucosinolates accounted for about 67.0% in rutabaga, 87.5% in green cauliflower and 90.6% in purple cauliflower. The lowest indole glucosinolate content was found in green (7.9%) and purple (8.4%) cauliflower (Table 1). When given in absolute values, the largest quantities of aliphatic and indole glucosinolates were present in purple cauliflower, then rutabaga and green cauliflower: 7.5 and 0.70 μmol, about 4.9 and 0.66 μmol, and 4.8 and 0.41 μmol per g dry matter of the raw material, respectively (Table 1).

In the examined raw *Brassicas*, the highest quantities of sinigrin (SIN) and glucoraphanin (GRA), constituting 58.4% of total aliphatic glucosinolates, were detected in purple cauliflower; green cauliflower contained lower amounts, i.e. 27.4%, while rutabaga had only 0.87% of the total amount of this group (Table 1).

The largest significant ($p \leq 0.05$) amounts of sinigrin and glucoraphanin (SIN/GRA), expressed in absolute values, were found in purple cauliflower (4.37 μmol/g dry matter), i.e. 71.2% more than the content of the above constituents in green cauliflower (1.26 μmol/g dry matter) and as much as 88.1% of the absolute amount determined in the raw rutabaga (0.05 μmol/g dry matter) (Table 1).

The second group of cancer-preventive hydrolysis products, glucobrassicins (GBS), is a significant part of the indole glucosinolates present in *Brassica* vegetables (Cieślik, Leszczyńska, Filipiak-Florkiewicz, Sikora, & Pisulewski, 2007). Of the indole GLS, glucobrassicin had the largest share in this group of compounds: 60.1% in green cauliflower green, and then 42.7% in purple cauliflower, whereas in rutabaga GBS amounted to only 3.0% of the total content of indole glucosinolates. When calculating the values per 1 g dry matter, the glucobrassicin (GBS) amounts were as follows: highest: 0.30 μmol, in purple cauliflower; 0.24 μmol, in green cauliflower; and lowest: 0.02 μmol, in rutabaga. These results differ from the values reported by Volden et al. (2009), according to which sinigrin and glucoraphanin (SIN/GRA) together constituted 27.2 and 56.7% of aliphatic glucosinolates in purple and green cauliflower,

respectively, while glucobrassicin (GBS) was 50.6 and 74.5% of indoles (Table 1).

3.1.3. *Isothiocyanates and indoles*

The content of isothiocyanates was highest in purple cauliflower (an absolute value of 6.4 μmol/g dry matter), followed by green cauliflower (0.82 μmol/ g dry matter) and rutabaga (0.21 μmol/g dry matter). As for total indoles, prior to heat treatment, green cauliflower contained the most of these constituents (0.33 μmol/g dry matter), then purple cauliflower (0.12 μmol/g dry matter) and rutabaga (0.05 μmol/g dry matter). Green cauliflower was also characterized by the highest level of indole-3-carbinol (0.26 μmol/g d.m.) (Table 2).

All the samples were ground after freezing and stored until analyses. Grinding was performed in order to obtain more representative results, while tissue disruption due to this operation allowed the hydrolysis of glucosinolates by myrosinase. This is the case because determination of the glucosinolates content is carried out after initial freezing of samples and their storage or after freezing and subsequent lyophilization. Freezing causes tissue damage in vegetables due to the growth of water crystals as well as cell disintegration, particularly when the process takes a long time. Moreover, it often happens that vegetables are ground and shredded before freezing (also after lyophilization) in order to assure sample representativeness. In addition, when blanching (or any other method of enzyme deactivation) is not applied, myrosinase remains active and causes hydrolysis of glucosinolates during thawing of samples. Furthermore, glucosinolates could undergo further degradation during the sample preparation for analyses (i.e. weighting and adding an internal standard) (Sosińska & Obiedziński, 2011).

Glucosinolates are very stable water-soluble precursors of isothiocyanates, and are typically present in fresh plants at much higher levels than their cognate isothiocyanates. Under carefully controlled conditions designed to extract glucosinolates and isothiocyanates completely, while preventing myrosinase activity, some fresh plants have been shown to almost exclusively contain glucosinolates. The relatively non-reactive glucosinolates are converted to isothiocyanates upon wounding of the plant, mastication of fresh plants or as a result of tissue damage caused by bruising or freeze during cultivation, harvest, shipping or handling (Fahey, Zalcmann, & Talalay, 2001).

Bioavailability of the isothiocyanates obtained from fresh and cooked (steamed) broccoli was compared by measuring the total urinary excretion of isothiocyanate equivalents within a period of 24 h in a group of 12 male subjects. Both kinds of broccoli preparations contained the same amount of isothiocyanate equivalents per g, and after consumption there was no significant difference in total glucosinolates. Analysis of the urinary excretion of isothiocyanates revealed that their bioavailability from fresh broccoli was ca. three times higher than from those cooked (Conaway et al., 2000; Latté et al., 2011). This along with the anticipated higher chemopreventive activity might be the reason for eating preventively raw broccoli by health-conscious people. When consumed raw, broccoli contains both the active myrosinase and epithiospecifier protein and the glucosinolates. Therefore, the reaction can continue in the gastrointestinal tract (Johnson, 2002; Latté et al., 2011). The quantitative and qualitative changes in the profile of products obtained from the glucosinolates hydrolysis are strictly connected with variability of the glucosinolates and the content of other food constituents. In view of the above, the pro-health effect observed after consumption of *Brassicas* may differ considerably. Therefore, further studies should focus on an influence of different factors not only on the content of glucosinolates but also on the formation of their breakdown products. Analysis of the results could be a problem because glucosinolate–myrosinase system has not yet been fully investigated. It is known that myrosinase-catalysed hydrolysis (when plant tissue is damaged) releases "free" glucosinolates and the enzyme. Moreover, myrosinase or rather a group of thioglycosides of different masses ranging 65–75 kDa and various susceptibility to environmental factors could be encoded by different genes (around 20 different genes in rape) depending on the stage of development and the morphological part of a plant. Activity of the enzyme is additionally influenced by the presence of myrosinase-binding proteins (MBPs) in vegetable tissue (Kissen, Rossiter, & Bones, 2009; Sosińska & Obiedziński, 2011).

3.2. *Changes in glucosinolates level due to thermal degradation and leaching*

Processing methods could result in a depletion of naturally occurring phytochemicals in *Brassica* vegetables. In some cases, however, their content remains unchanged and sometimes processing can even lead to the formation of novel compounds with biological activity (Volden et al., 2009). On the other hand, the inactivation of myrosinase by cooking does not necessarily mean that the glucosinolates breakdown is stopped. Glucosinolates are not inert substances, but are degraded

Table 2 – Content of decomposition products of glucosinolates in fresh and cooked green cauliflower, purple cauliflower and rutabaga (μmol/g d.w.).

Compounds	Green cauliflower fresh	Green cauliflower cooked	Purple cauliflower fresh	Purple cauliflower cooked	Rutabaga fresh	Rutabaga cooked
Indole-3-carbinol	0.263 ± 0.00^{d}	0.098 ± 0.00^{c}	0.094 ± 0.00^{c}	0.030 ± 0.00^{b}	0.035 ± 0.00^{b}	0.020 ± 0.00^{a}
Indole-3-acetic acid	0.060 ± 0.01^{b}	0	0.079 ± 0.00^{c}	0	0.010 ± 0.00^{a}	0
Indole-3-acetonitrile	0.004 ± 0.00^{a}	0.069 ± 0.00^{d}	0.009 ± 0.00^{b}	0.01 ± 0.00^{c}	0.003 ± 0.00^{a}	0.099 ± 0.00^{e}
Diindolylmethane	0.001 ± 0.00^{a}	0	0.004 ± 0.00^{b}	0	0	0
Total indoles	0.330 ± 0.01^{e}	0.170 ± 0.00^{c}	0.186 ± 0.00^{d}	0.045 ± 0.00^{a}	0.049 ± 0.00^{a}	0.119 ± 0.00^{b}
Total isothiocyanates	0.822 ± 0.10^{b}	0.730 ± 0.04^{b}	6.374 ± 0.21^{e}	3.674 ± 0.09^{d}	0.238 ± 0.02^{a}	1.022 ± 0.04^{c}

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 ($p \leq 0.05$).

depending on various other factors such as strong acid or base or different types of salts (Hanschen et al., 2014).

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. In the case of green and purple cauliflower, it has been observed that indole glucosinolates are more sensitive to cooking, since losses due to cooking amounted to 80.0 and 88.4%, respectively, including the glucobrassicin (GBS) content which fell by 82.3 and 93.6% compared to raw vegetables. Simultaneously, in green and purple cauliflower, losses in aliphatic glucosinolates were slightly lower (about 67.0%); the sinigrin and glucoraphanin (SIN/GRA) levels decreased by 60.8 and 71.6%, respectively, compared to the vegetables before heat treatment. In rutabaga, the contents of indole and aliphatic glucosinolates fell by 23.1%, and 2.3%, respectively, in comparison to raw vegetables. It is worth noting that certain aliphatic glucosinolates in rutabaga exhibited a significant ($p \leq 0.05$) increase compared to raw vegetables. These were progoitrin (PRO), about 5.6%; glucoalyssin (GAL), about 84.2%; glucoiberverin (GIV), about 24.9%; and gluconapoleiferin (GNF), about 38.9%. Similarly, despite losses in the total amount of indole glucosinolates, the content of glucobrassicin (GBS) after heat treatment was found to be significantly higher ($p \leq 0.05$), i.e. 190%, compared to raw vegetables (Table 1).

Various processes during heat treatment and subsequently (during storage or preparation of samples) may have an impact on the content of glucosinolates in vegetables. The distribution of the glucosinolates that have been examined varies among plant organs, with both quantitative and qualitative differences between roots, leaves, stems and seeds. Major determinants of the qualitative and quantitative glucosinolate composition of plants are plant age, environmental factors such as soil fertility, pathogen challenge, wounding or plant growth regulators. These factors have significant effects on the levels of specific glucosinolates in growing plants and may affect distribution among plant organs (Fahey et al., 2001).

A similar decrease in total glucosinolate content due to cooking was reported in the model study by Tiwari et al. (2015). The authors found that 20-minute cooking *Brassicas* reduced the total glucosinolates content by approx. 50–76%; they also emphasized that as much as 80% of this amount is due to leaching and only 20% results from the high temperatures. According to Cieślik et al. (2007), cooking for 10–15 min led to losses in glucosinolates in selected *Brassica* vegetables (Brussels sprouts, white and green cauliflower, broccoli and kale); the losses ranged from 35.3 to 72.4%. McNaughton and Marks (2003) compared the glucosinolates content in raw, cooked, or boiled vegetables and found losses of glucosinolates in a range of 18.1–59.1%, with the highest loss resulting from boiling. Song and Thornalley (2007) demonstrated a progressive decrease in total glucosinolates content after boiling for 30 min of 58% in Brussels sprouts, 65% in green cabbage, 75% in cauliflower and 77% in broccoli. Interestingly, steaming, microwaving and stir-stir fry cooking had little effect on the total glucosinolate analyte amounts of *Brassica* vegetables under the conditions studied by these authors. On the other hand, the mean losses due to traditional cooking in water observed by Gliszczyńska-Świgło et al. (2006) were lower, i.e. 48%; a similar 41.0% reduction in cooked broccoli was noted by Yuan, Sun, Yuan, and Wang (2009), while according to the authors of an older publication mean losses in cabbage subjected to boiling for 10 minutes were lower and amounted to 28% (Sones, Heaney, & Fenwick, 1984). It is possible that these differences result from the different methods applied to determine these compounds. Volden et al. (2009) reported significant reductions in total glucosinolates in all processed cultivars, ranging from 18 to 22% in steamed, 30–52% in blanched and 46–61% in boiled cultivars. Total aliphatic glucosinolates were reduced by 42–60% in boiled florets of five different varieties of cauliflower. Reductions in total indole glucosinolates were quite similar: boiled 49–62%. Thus, the effects of thermal treatment were most severe for boiling, less so for blanching and least for steaming. In the present study, total glucosinolate losses in cooked cauliflower were a little higher, an average of 68.2%; higher decreases were also found in aliphatic and indole glucosinolates. Tiwari et al. (2015) concluded that the inactivation of enzymes occurs predominantly during prolonged cooking (i.e. > 3 min) of *Brassica* and thus leads to a severe degradation of the glucosinolates derivatives. The study by Korus et al. (2014) found that boiling kale leaves caused a 42.0% mean decrease in total glucosinolates content compared to the fresh material. Indole glucosinolates were more sensitive to boiling (51.0%) than aliphatic glucosinolates (38.0%). Similar trends were observed in this study.

The increase in the levels of certain glucosinolates in rutabaga could be caused by the completely different morphology of this plant (root) compared to that of cauliflower, so such differences may result from the different structure of the two vegetables. The glucosinolate–myrosinase system has not been yet fully explored, so the results of tests carried out for crops other than rape and horseradish are not clear. Sosińska (2005) observed a 55% higher content of total glucosinolates in Brussels sprouts after 4 min of conventional boiling than in raw vegetables. Similar conclusions were drawn by Verkerk and Dekker (2004), who found 78% more glucosinolates in red cabbage after microwave treatment than in the raw vegetable. Roqué-Sala (2005) also determined about 86% more glucosinolates in Brussels sprouts after microwave treatment as compared to raw vegetables. Miglio, Chiavaro, Visconti, Fogliano, and Pellegrini (2008) also reported the highest concentration of glucosinolates in steam boiled broccoli (93.4 µmol/g dm), in comparison to raw (71.4) and conventionally boiled ones (29.3).

The differences in thermal degradation of glucosinolates may result from the presence of specific plant components which negatively influence the glucosinolate stability. The higher content of glucosinolates in plant tissue results in their higher sensitivity to thermal degradation. During cooking some components, including glucosinolates, migrate into water and become diluted. It is possible that there is another mechanism that determines the variations in thermal stability of glucosinolates in different environments.

3.2.1. *Degradation products of glucosinolates*

The process of cooking caused a significant ($p \leq 0.05$) reduction in the indole-3-carbinol (I3C) in green and purple cauliflower and rutabaga, 37.7, 68.0 and 42.9%, respectively, compared with raw vegetables. In the remaining raw vegetables, except for rutabaga, 3,3′-diindolylmethane (DIM) was determined in trace amounts and heat treatment applied led

to a 100% reduction in its presence. In all the analysed *Brassica* vegetables, a significant increase ($p \leq 0.05$) in indole-3-acetonitrile (I3ACN) due to cooking was registered compared to raw vegetables: 12.4% (purple cauliflower), 72.5% (green cauliflower) and up to 2.9% (rutabaga). As a result of cooking, the total indoles content decreased in green and purple cauliflowers by 48.5 and 75.8%, respectively, and increased by 142.9% in rutabaga, compared to vegetables before treatment. For isothiocyanates (ITC sum), there were similar trends. Cooking meant that their concentration decreased significantly ($p \leq 0.05$) in green and purple cauliflowers (by 11.0 and 42.4%, respectively) and increased (by 329.4%) in rutabaga in comparison with raw vegetables (Table 2).

Nitriles and isothiocyanates are the main products of glucosinolate degradation. Nitriles are present even during heat treatments at temperature higher than 100 °C and hardly degrade any further. Therefore, their losses during cooking predominantly occur because of their volatility. On the other hand, isothiocyanates contain a very electrophilic carbon atom and readily react with nucleophiles such as hydroxyl, amino, or thiol groups, thereby forming *O*-thiocarbamates, thiourea derivatives, or dithiocarbamates, respectively. The composition of breakdown products depends on pH values during the reaction (Hanschen et al., 2014; Naczk, Shahidi, Diosady, & Rubin, 1986; Shahidi & Gabon, 1989; Zhang & Talalay, 1998).

Jiao, Yu, Hanker, Low, and Chung (1998) conducted studies on isothiocyanate content in selected *Brassica* vegetables before and after cooking. Of the 82 *Brassicas* samples analysed, only in three were there isothiocyanates after cooking. These quantities were very small compared with the samples that were cooked and then underwent myrosinase-catalysed hydrolysis. According to the findings of other authors, no glucosinolate breakdown products were present in cooked *Brassica* vegetables (De Vos & Blijleven, 1988). These results only partially agree with those obtained in this study, since cooking led to declines in isothiocyanates in green and purple cauliflowers; however, in rutabaga, this resulted in a more than 3-fold increase compared to their content in the raw vegetable. As for the glucosinolates breakdown products, their amounts and profile depend not only on glucosinolate–myrosinase system, but also on some additional specifier proteins. Myrosinase-catalysed glucosinolates degradation leads to the formation of unstable intermediate thiohydroxamate-*O*-sulphonate which, in the absence of specifier proteins, is converted spontaneously to the most desirable compounds, i.e. isothiocyanates and indoles exhibiting the strongest biological activity. However, the catalytic activities of these proteins, which apart from myrosinase participate in the glucosinolates degradation process, promote formation of other less bioactive compounds. Rungapamestry, Duncan, Fuller, and Ratcliffe (2006) observed the reduction of myrosinase activity during cabbage steaming or microwaving; however, this enzyme still retained some activity. The authors also observed parallel changes in the profile of the glucosinolates degradation products depending on the type of cooking and the length of treatment. During cabbage steaming, the rate of glucosinolates conversion to cyanoepithioalkanes decreased and the content of isothiocyanates increased. The authors suggested that additional protein factors – epitiospecific proteins (ESP) – in raw and near-raw cabbage, despite possessing the highest myrosinase activity, might have blocked the rearrangement of thiohydroxamate-*O*-sulphonate to isothiocyanates. Upon longer cooking, probably due to ESP denaturation, production of isothiocyanates increased proportionally to the reduction in the cyanoepithioalkane formation, which is consistent with our findings. Apparently, similar to cabbage, specifier proteins in rutabaga are less thermally resistant than myrosinase, which explains the higher content of isothiocyanates and indoles (spontaneously formed from indolic isothiocyanates) in cooked rutabaga compared to the raw material. This increase could be due to the different morphology of rutabaga, where the root is more compact and resistant to damage compared to a cauliflower. The extent of losses occurring during cooking depends not only on the manner in which this process is performed (time, temperature), but also on the varietal characteristics of the raw material, which can determine the chemical composition of glucosinolates. It is highly likely that, in the case of rutabaga, the pre-processing meant that the enzyme myrosinase was released to a lesser degree and only after the application of cooking did enzyme activation take place; longer heating of the water and longer maintenance of the enzyme at its optimal temperature (i.e. until water reached 90 °C) activated the enzyme and resulted in the formation of glucosinolate breakdown products. Thus, boiling for more than a minute, or steaming for more than 4 or 5 min, will lead to a loss of myrosinase activity (Rungapamestry, Duncan, Fuller, & Ratcliffe, 2007; Tang et al., 2013). It has been reported that cooking reduces isothiocyanate exposure from *Brassica* vegetables by 60–90% (Conaway et al., 2000; Getahun & Chung, 1999; Shapiro, Fahey, Wade, Stephenson, & Talalay, 1998). The increase in the sum of indoles and isothiocyanates in dry matter of the cooked rutabaga could result from more efficient leaching of other constituents soluble in boiling water, which in turn increases the proportion of these constituents in 1 g dry matter.

Sosińska and Obiedziński (2011) observed the same tendency in the growth of indole-3-acetonitrile (I3ACN) under the influence of the cooking process. The increase was directly proportional to the cooking time (5, 10, 20 min). The authors explained that the increase in these compounds was most likely due to the low activity of epithiospecific proteins in the analysed vegetable varieties. The authors also suggested that when the myrosinase underwent denaturation during the boiling of vegetables, the glucobrassicin might have undergone further transformations, but nitriles could also have formed through non-enzymatic decomposition of glucosinolates. Ciska, Verkerk, and Honke (2009) reported that after 40 and 50 min of boiling of fermented cabbage, the total content of 3,3′-diindolylmethane was 6-fold higher than that in uncooked vegetable. 3,3′-Diindolylmethane synthesis proceeded within the plant tissue. After 10 min of boiling, the content of free indole-3-carbinol and indole-3-acetonitrile was about 80% of that of uncooked cabbage.

The conditions of vegetable thermal processing can significantly affect the amount of constituents delivered to the gastrointestinal tract with a portion of food (Tiwari et al., 2015). Consumption of raw vegetables can lead to exposure of the organism to high levels of nitriles, while their gentle and shorter cooking can increase the amount of isothiocyanates operating in upper sections of the gastrointestinal tract at a high, from the biological point of view, level of concentration. Cooking, when too intense, prevents the formation of isothiocyanates

and allows consumption of glucosinolates in unchanged form; these can be degraded to isothiocyanates or other constituents by bacterial flora in the colon, which is characterized by quite a low level of efficiency (Tang et al., 2013).

However, if these vegetables are cooked, then it would be better to consume together with cooking water, for example as soups, since such water may contain glucosinolates and their breakdown products (Sosińska & Obiedziński, 2011). Further research is needed to increase knowledge regarding the bioavailability of glucosinolates and their decomposition products from different vegetables, and to confirm the effects of various domestic cooking methods on the concentration and structure of these compounds.

4. Conclusions

Of the analysed *Brassica* vegetables, the best source of aliphatic and indole glucosinolates was purple cauliflower, while the smallest amounts of aliphatic and indole glucosinolates were found in green cauliflower. The largest amount of active anti-cancer constituents, such as sinigrin and glucoraphanin, was in purple cauliflower, which contained more, on average 79.6%, than green cauliflower and rutabaga. In every case in cooked vegetables significant losses in total glucosinolate content were detected, ranging from 6.6% in rutabaga to approx. 69.0% in both cauliflowers. The process of cooking reduced the sum of indoles and isothiocyanates in green and purple cauliflower by approx. 62.5 and 26.7%, respectively, but increased their amount in rutabaga by 236.1%. The environment in cells or cell compounds of cauliflower and rutabaga can exert influence on the thermostability of glucosinolates and their breakdown products. The above analysis indicates that further studies should include the conversion path of glucosinolates during digestion, including stability and type of decomposition products formed under different conditions; the role of the human microflora on the bioconversion of glucosinolates; the composition of the food matrix as an effect of cooking and its modulating role in the conversion of glucosinolates; and finally total content of useful degradation products of glucosinolates capable of being absorbed in the digestive tract. The overall effect of vegetables containing glucosinolates can be accurately assessed only by analysing the impact of the conditions of cooking on the glucosinolate content and activity of myrosinase.

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