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EFFECT OF HYDROTHERMAL PROCESSING ON PHENOLIC ACIDS AND FLAVONOLS CONTENTS IN SELECTED BRASSICA VEGETABLES

Elżbieta Sikora[1]✉, Ewa Cieślik[2], Agnieszka Filipiak-Florkiewicz[2], Teresa Leszczyńska[1]

[1]Department of Human Nutrition, Agricultural University in Krakow
Balicka 122, 30-149 Cracow, **Poland**
[2]Malopolska Centre of Food Monitoring and Certification
Balicka 122, 30-149 Cracow, **Poland**

ABSTRACT

Background. Commonly occurring diseases can have the origin in oxidative processes ongoing in the human body. Vegetables of *Brassicaceae* family are the essential sources of natural antioxidants, especially phenolic compounds, in the human diet. The research was aimed to estimate the content of phenolic compounds in selected vegetables and their quantity changes during hydrothermal processes.
Material and methods. The vegetables subjected to analysis were: kale, broccoli, Brussels sprouts, and white and green cauliflower. The fresh and processed (blanched, cooked, frozen, cooked after freezing) vegetables were freeze-dried. The levels of phenolic acids and flavonols by HPLC method were estimated.
Results. The presence of derivatives of hydroxycinnamic acid, mainly of caffeic acid, p-coumaric acid, sinapic acid and of flavonols – kaempferol, and in smaller amounts of quercetin was found. The largest amounts of above components were present in kale (total 94.4 mg·100 g^{-1} of fresh matter), whereas the smallest amounts were found in white and green cauliflower – 3.6 mg·100 g^{-1} f.m. and 3.03 mg·100 g^{-1} f.m., respectively. The applied technological processes contributed to lover amounts of all tested compounds depending on the process and the vegetable kind. The biggest loses, up to 70-80%, took place during cooking of raw and previously frozen vegetables.
Conclusions. Analysed *Brassicaceae* were characterized by high contents of the investigated flavonoids. The best source of those compounds was kale whereas the smallest amounts of searched components were presented in cauliflowers. The used hydrothermal processes led to losses of searched compounds.

Key words: *Brassica* vegetables, phenolic acids, flavonols, hydrothermal processing

INTRODUCTION

Commonly occurred diseases, such as cardiovascular dysfunction, some kinds of tumors and few other serious illnesses can have the origin in oxidative processes ongoing in the human body. The intensity of those processes, evoked by different environmental factors is sometimes larger than natural ones. Overproduction of reactive forms of oxygen can lead, in some circumstances, to oxidative stress which ruins body cells and tissues [Parthasarathy et al. 2001, Jain 2006]. It was shown that enzymatic – antioxidant defending body system could be efficiently supported by antioxidant substances provided by proper diet, which may be of prophylactic importance [Arts and Hollman 2005, Mennen et al. 2005].

Fruits and vegetables are commonly known as the basic and most plentiful sources of natural components

✉rresikor@cyf-kr.edu.pl

with antioxidant activity in human diet. Ascorbic acid, carotenoids, tocopherols, some mineral compounds and phenolic compounds show such properties. So the diet rich in fruits and vegetables has significant pro-healthy properties [Steinmetz and Potter 1996, Lampe 1999, Prior 2003, Rohrmann et al. 2007].

The phenolic acids such as hydroxybenzoic and hydroxycinnamic and their derivatives and some flavonoids – flavonols, flavones, isoflavones, anthocyanidins and flavanols belong to natural phenolic compounds. The presence of those components in plants' world is much differentiated. Berry fruits are the most plentiful sources of anthocyanidins, while citrus fruits contain big quantities of flavonones, soy bean – isoflavones, coffee and tea – chlorogenic acid and catechins [Prior 2003, Manach et al. 2004].

Vegetables of *Brassicaceae* family are the essential sources of phenolic compounds in the human diet. They contain also derivatives of hydroxycinnamic acid – caffeic, chlorogenic, ferulic, and synapic as well as flavonols (kaempferol derivatives, quercetin derivatives) and anthocyanins (red cabbage) [Vallejo et al. 2003 a, Heimler at al. 2006, Podsędek et al. 2006]. Pro-healthy properties of the products are determined not only by the quantity and quality of polyphenols contents, but also by the yield of production and consumption [Llorach et al. 2003 a, Chun et al. 2005, Cieślik et al. 2006].

The research was aimed to estimate the content of phenolic compounds in selected vegetables and their quantity changes during hydrothermal processes that the vegetables were submitted to before consumption.

MATERIAL AND METHODS

The vegetables subjected to analysis were: kale (*Brassica oleracea* var. *acephala*, cv. Winterbor), broccoli (*Brassica oleracea* var. *botrytis italica*, cv. Sebastian), Brussels sprouts (*Brassica oleracea* L. var. *gemmifera*, cv. Maczuga), and cauliflower (*Brassica oleracea* var. *botrytis*) – white cauliflower (cv. Rober) as well as green cauliflower (cv. Amphora). The vegetables were cultivated in the Polan Plant and Horticultural Seed Production Centre in Krakow, Poland.

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

The methodology of HPLC estimation [Oszmiański and Wojdyło 2005]. The lyophilised samples weighing 0.5 g were hydrolysed enzymatically with the mixture of enzymes (Drum pectinase 263; β-glucozydase, SIGMA; Hesperydynase, SIGMA; Sulfatase type H-2, SIGMA) diluted in 5 ml of citric buffer with pH 5.5. The samples were then treated with mixture of enzymes and then incubated in water bath – temp. 40°C, during 1 hour; left in darkness for 20 hours, in ambient temperature, to let the enzymatic hydrolysis occur. After this, the samples were treated with 5 ml of pure methanol and left for 10 minutes in ultrasound bath. The disintegrated samples were centrifuged and analysed with HPLC.

The phenolic compounds were estimated by HPLC--high performance liquid chromatography with liquid chromatograph with diode detector Merck-Hitachi L-7455. The detector was cooperating with L-7100 pump and with reagents mixing system D-7000 HSM Multisolwent Delivery System. The apportionment was performed at the column LiChroCART® 125-3 Purospher® RP-18 (5 μm) Merck, thermostated in 30°C. As an eluent the 80% acetonitryl solution in 4.5% formic acid (reagent A) and a 2.5% acetic acid (reagent B) were used with flow of 1ml/min. Concerning the gradient the reagent A was risen linearly to 7th minute from 0% to 15%, then to 15th minute to 20% and in 16th minute to 100%. After 10 minutes of washing up

of column the concentration of reagent A was lowered to 0%, for 10 minutes, to stabilize the column before next injection of sample. During assessment all dilutions were degassed with Merck apparatus.

Registration was done at λ = 320 nm (phenolic acids), 340 nm (flavones), and 360 nm (flavonols). Each compound was identified basing on spectra from 200 nm to 600 nm and on the retention times and compared to standards produced by EXTRASYNTHESE (France).

The results were analysed with Statistica v.8 (Statsoft LTD., Poland) licensed program. The one and two factor analysis of variance were performed. The significance of differences between means was estimated with post hoc Duncan test with $p < 0.05$ and with $p < 0.01$.

RESULTS AND DISCUSSION

Estimated vegetables differed concerning the sum of assessed phenolic compounds and also participation of individual components. The highest amount was obtained in kale (94.4 mg·100 g^{-1} f.m.) and slightly less in Brussels sprout – 26.0 mg·100 g^{-1}. Also high total amount of all assessed compounds was presented in broccoli (14.48 mg·100 g^{-1}) whereas white and green cauliflower contained 3.6 and 3.03 mg·100 g^{-1} of those compounds, respectively (Table 1).

The sum of phenolic acids in kale was 59.59 mg·100 g^{-1} f.m. Over 40% share of those acids was for caffeic acid, and almost 50% share – in equal parts p-coumaric acid and sinapic acid. Sinapic acid was in prevalence in Brussels sprout, broccoli and green cauliflower.

Applied hydrothermal treatment to vegetables caused changes in the amount of phenolic compounds. The changes were dependent on the kind of process and vegetable species (Table 1). The largest loses up to 70-80% took place during cooking of assessed fresh and previously frozen vegetables. Especially large loses of flavonoids were found in kale what could be connected with huge level of vegetable disintegration.

The applied technological processes caused bigger flavonols lost than phenolic acids (Table 2). Only in case of kale the loss of phenolic acids and flavonols was similar. The very low retention was observed for luteolin and apigenin in assessed green cauliflower.

The recent researches on levels and kinds of polyphenols in *Brassicaceae* performed by other authors showed that the amounts of hydroxycinnamic acid (caffeic acid, chlorogenic acid, sinapic acid) in vegetables was comparable to results in the present [Sakakibara et al. 2003, Vallejo et al. 2003 a, b, Llorach et al. 2003 b, Nilsson et al. 2006, Heimler et al. 2006, Mattila and Hellström 2007]. There were some differences concerning the content of few acids, which could be caused by the methodological factors i.e. whether the water or ethanol extract were assessed and kind of vegetable itself or its growing up conditions. Generally biological material is characterized by generally great differentiation because the content of single component can be modified by many factors.

Among flavonols mainly kaempferol and in lesser amounts quercetin were identified in tested *Brassica* vegetables. The highest amounts of those compounds

Table 1. The sum of investigated polyphenols in fresh and processed *Brassica* vegetables, mg·100 g^{-1} fresh matter

Sample	Broccoli	Brussel sprouts	Kale	Cauliflower	Green cauliflower	ANOVA	$NIR_{(p<0.01)}$
Fresh	14.46 ±0.03	26.1 ±0.10	94.97 ±0.21	3.58 ±0.01	3.04 ±0.03	$9.64 \cdot 10^{-77}$	0.023
Cooking	9.11 ±0.01	19.1 ±0.00	26.26 ±0.03	0.93 ±0.02	1.56 ±0.03		
Blanching	9.77 ±0.02	20.3 ±0.21	49.78 ±0.01	2.11 ±0.01	3.06 ±0.04		
Freezing	8.98 ±0.01	12.5 ±0.01	48.89 ±0.11	1.53 ±0.01	2.79 ±0.01		
Cooking after freezing	4.94 ±0.04	3.2 ±0.00	13.42 ±0.00	0.52 ±0.00	0.77 ±0.02		
ANOVA	$1.34 \cdot 10^{-68}$					$5.16 \cdot 10^{-68}$	0.005
$NIR_{(p<0.01)}$	0.023						

Table 2. Phenolic acids and flavonoids in fresh and processed *Brassica* vegetables, mg·100 g^{-1} fresh matter

Sample	Caffeic acid	Ferulic acid	p-Coumaric acid	Sinapic acid	Quercetin	Kaempferol	Isorhamnetin	Apigenin	Luteolin
Broccoli									
Fresh	0.4 b	1.98 e	1.95 d	2.95 c	2.09 c	3.60 d	1.49 c	–	–
Cooking	0.42 b	1.21 b	1.43 b	2.16 b	1.03 b	1.55 b	0.50 b	–	–
Blanching	0.46 c	1.81 d	1.48 bc	2.92 c	1.17 b	1.93 c	0.58 b	–	–
Freezing	0.43 b	1.39 c	1.57 c	2.17 b	1.09 b	1.59 b	0.53 b	–	–
Cooking after freezing	0.24 a	0.64 a	0.79 a	1.41 a	0.65 a	0.99 a	0.30 a	–	–
Brussel sprouts									
Fresh	3.98 d	2.28 d	4.24 d	13.57 e	0.59 d	1.40 d	–	–	–
Cooking	4.14 e	1.71 b	3.52 c	8.63 c	0.35 b	0.78 b	–	–	–
Blanching	2.94 b	1.85 c	3.52 c	10.37 d	0.41 c	1.18 c	–	–	–
Freezing	3.03 c	1.79 bc	3.34 b	2.85 b	0.41 c	1.10 c	–	–	–
Cooking after freezing	0.63 a	0.40 a	0.82 a	1.01 a	0.09 a	0.20 a	–	–	–
Kale									
Fresh	25.95 e	5.76 d	14.49 d	13.39 d	8.48 e	26.90 d	–	–	–
Cooking	6.26 b	1.78 b	3.87 b	4.27 b	2.48 b	7.59 b	–	–	–
Blanching	11.57 c	3.29 c	8.55 c	9.44 c	4.26 d	12.67 c	–	–	–
Freezing	12.10 d	3.16 c	8.22 c	9.08 c	3.92 c	12.40 c	–	–	–
Cooking after freezing	2.83 a	1.03 a	1.90 a	2.15 a	1.31 a	4.20 a	–	–	–
Cauliflower									
Fresh	0.53 d	0.30 d	0.72 d	0.70 d	0.33 c	1.00 c	–	–	–
Cooking	0.08 b	0.16 b	0.28 b	0.24 b	0.04 a	0.13 a	–	–	–
Blanching	0.24 c	0.20 b	0.60 c	0.62 c	0.15 b	0.31 b	–	–	–
Freezing	0.21 c	0.17 b	0.17 ab	0.61 c	0.08 a	0.29 b	–	–	–
Cooking after freezing	0.04 a	0.09 a	0.10 a	0.15 a	0.04 a	0.10 a	–	–	–
Green cauliflower									
Fresh	0.20 b	0.25 c	0.59 d	1.36 c	0.06 b	0.07 b	–	0.14 b	0.37 c
Cooking	0.16 b	0.17 b	0.26 b	0.84 b	0.02 a	0.02 a	–	0.03 a	0.05 a
Blanching	0.30 c	0.24 c	0.49 c	1.65 d	0.04 ab	0.03 a	–	0.04 a	0.26 b
Freezing	0.28 c	0.24 c	0.49 c	1.70 d	0.02 a	0.03 a	–	0.00 a	0.03 a
Cooking after freezing	0.05 a	0.10 a	0.12 a	0.45 a	0.01 a	0.03 a	–	0.00 a	0.01 a

Within each vegetable in the some column values followed by the same letter are not significantly different ($p < 0.05$).

were presented in kale (35.38 mg·100 g^{-1} f.m.). Five times less amount of flavonols was found in broccoli (7.18 mg·100 g^{-1} f.m.) whereas these compounds were in relatively small amounts in all other estimated vegetables. What is more, in green cauliflower also flavones, like luteolin and apigenin, were found (Table 2).

Other authors pointed that mainly glycosides of kaempferol and its combinations with different hydroxycinnamic acids were present in *Brassicaceae* [Price et al. 1998, Llorach et al. 2003 a, b, Heimler et al. 2006]. Sakakibara et al. [2003] reported the presence of luteolin in broccoli and Bahorun et al. [2004] – presence of apigenin in cauliflower. Comparing the literature data it can be concluded that the kind and amount of flavonoids in *Brassicaceae* depends, to the great extent, on the species and hybrid of vegetable and conditions of cultivation and also on methods of their estimation.

In case of all vegetables submitted to cooking or blanching process the significant losses of each estimated compound were observed in comparison to raw material, whereas the freezing process did not cause any significant changes in comparison to blanched vegetable. Cooking of blanched vegetable caused further mostly significantly reduction in phenolic acids and flavonoids contents in comparison to raw material and to previous (initial) technological process (Table 2).

Other authors also observed the decline of flavonoids content during cooking of Brassica vegetables. Vallejo et al. [2003 b] found that during conventional cooking of broccoli the amount of caffeic acid derivatives lowered up to 62% in comparison to the level presented in fresh vegetable, and for sinapic and ferulic acids derivatives up to 51%. Price et al. [1998] observed the changes of kaempferol and quercetin' glycosides stated that the retention of those compounds was at level of 14-28%. Big decrease of quercetin and kaempferol was confirmed also by Ewald et al. [1999] for cooked onion, green bean and peas.

Many authors described simultaneously that during blanching of vegetables and during their microwave and/or damp cooking the losses of estimated flavonoids were smaller than during conventional cooking [Price et al. 1998, Ewald et al. 1999, Vallejo et al. 2003 b, Gębczyński and Lisiewska 2006, Gębczyński and Kmiecik 2007, Viňa et al. 2007, Olivera et al. 2008].

According to Olivera et al. [2008] flavonol glycosides are presented in epidermal layers of plant, in hydrophilic zones. They are well soluble in water and this is the reason of their big losses during cooking, in large amounts of water. From the above it can be concluded that *Brassica* vegetables should be cooked in the smallest amount of water possible, or with hot steam or microwaved application, which allows the preservation to the greater extent the assessed bioactive compounds to a greater extent and preserves better the health value of vegetables at the same time.

Also the influence of level disintegration of assessed vegetable was affirmed on flavonoids amounts losses during cooking – for kale the losses were larger than for all others vegetables. There were also generally higher losses observed in flavonols content than phenolic acids what could be connected with better solubility of those acids in fats than in water [Hunter and Fletcher 2002].

The estimations of frozen vegetables previously blanched showed lower content of the compounds in comparison to fresh and blanched vegetables. Only for broccoli and kale losses were very low at the second type of treatment. Other authors also observed less content of phenolic compounds in frozen vegetables in comparison to fresh vegetables [Hunter and Fletcher 2002, Ninfali and Bacchiocca 2003, Gębczyński and Lisiewska 2006, Gębczyński and Kmiecik 2007].

Simultaneously Ninfali and Bacchiocca [2003] and Viňa et al. [2007] accented the meaning of blanching conditions for retentions of above mentioned compounds. Observed flavonoids' decreases in frozen vegetables in comparison to blanched could probably also dependent on freezing process conditions and are probably caused by freezing out of water from the product.

CONCLUSIONS

The analysed *Brassicaceae* were characterized by high contents of flavonoids that contain derivatives of hydroxycinnamic acid and of flavonols, mainly kaempferol. The best source of those compounds was kale whereas the smallest amounts of searched components were presented in cauliflowers. The hydrothermal processes, to which all those vegetables are usually submitted, led to losses of the searched compounds,

which was influenced by the process kind, and level of vegetable disintegration and also of flavonoid kind.

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WPŁYW PROCESÓW HYDROTERMICZNYCH NA ZAWARTOŚĆ KWASÓW FENOLOWYCH I FLAWONOLI W WYBRANYCH WARZYWACH KAPUSTOWATYCH

STRESZCZENIE

Cel. Przyczyną wielu współczesnych chorób są procesy oksydacyjne zachodzące w organizmie człowieka. Warzywa kapustowate (*Brassicaceae*) są bogatym źródłem antyoksydantów, szczególnie związków fenolowych w diecie człowieka. Celem badawczym było oznaczenie zawartości związków fenolowych w wybranych warzywach kapustowatych oraz ich zmian w wyniku obróbki hydrotermicznej.
Materiał i metodyka. Materiałem badawczym były: jarmuż, brokuł, kapusta brukselska oraz biały i zielony kalafior. Warzywa świeże oraz poddane obróbce hydrotermicznej (blanszowanie, gotowanie, mrożenie po blanszowaniu, gotowanie po mrożeniu) były liofilizowane. Zawartość kwasów fenolowych i flawonoli w uzyskanym materiale oznaczono metodą HPLC.
Wyniki. W warzywach stwierdzono obecność pochodnych kwasu hydroksycynamonowego, głównie kwasu kawowego, p-kumarowego i sinapowego, a także flawonoli – kempferolu i kwercetyny, w mniejszej ilości. Najwięcej tych związków zawierał jarmuż (w sumie 94,4 mg na 100 g ś.m.), a najmniej kalafior o róży białej i o róży zielonej – odpowiednio 3,6 i 3,03 mg/100 g. We wszystkich warzywach odnotowano zmniejszenie zawartości badanych związków w wyniku zastosowanych procesów technologicznych. Największe straty, rzędu ok. 70-80%, nastąpiły w procesie gotowania warzyw surowych i uprzednio zamrożonych.
Wnioski. Badane warzywa charakteryzowały się dużą zawartością flawonoidów, na które składały się pochodne kwasu hydroksycynamonowego oraz flawonole, głównie glikozydy kempferolu. Najlepszym źródłem tych związków był jarmuż, natomiast najmniejsze ilości zawierały kalafiory. Procesy hydrotermiczne powodowały straty badanych związków.

Słowa kluczowe: warzywa kapustowate, fenolokwasy, flawonole, procesy wodno-termiczne

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