EFFECT OF CULINARY TREATMENT ON CHANGES IN THE CONTENTS OF SELECTED NUTRIENTS AND NON-NUTRIENTS IN CURLY KALE (*BRASSICA OLERACEA* VAR. *ACEPHALA*)

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ABSTRACT

Kale has a great nutritive value, very high antioxidant activity and pro-healthy potential. The level of phytochemicals and related bioactive compounds are strongly dependent on pre and postharvest stages of production chain (domestic or industrial). The study investigated changes in the levels of vitamin C, β-carotene, total polyphenols, antioxidant activity, degradation products of glucosinolates as well as nitrates and nitrites in the kale due to the conventional cooking process. As a result of cooking kale, a significant decrease was noted in the content of vitamin C, polyphenols, diindolilometan, indole-3-acetic acid and nitrate and nitrites, and a significant growth in the content of β-carotene, indole-3-carbinol, indole-3-acetonitrile, total indoles and total isothiocyanate compared with the raw vegetable. It has been found that kale, fresh and cooked, had similar antioxidative potential.

PRACTICAL APPLICATIONS

Brassica species are commonly occurring in a diet as an additive to meat dishes and other rich-in-fat products, which favor tumor transformation. Due to their high consumption and availability over the whole year, brassicas may potentially be a crucial element in chemoprevention of cancer. The weakness of vegetables is their seasonal availability and fact that they are perishable. During processing of these vegetables for food preparation the most commonly used technique is cooking. Food technology is focused on discovering and establishing the methods of food processing, which will least affect their chemical composition. It is very important to know how to optimize, e.g., conventional cooking process to preserve beneficial and bioactive compounds of vegetables.

INTRODUCTION

Brassicas belong to the species popular in China, Japan, India and European countries, where their cultivation rises steadily, accompanied by an increase in their supply and consumption. This is due to their taste, nutritional value and high content of the biologically active components as well as a large abundance of minerals, compared with other vegetable groups (Kusznierewicz *et al.* 2008; Hounsome *et al.* 2009; Pedras and Yaya 2010; Kapusta-Duch *et al.* 2012).

Kale (*Brassica oleracea* var. *acephala*), both fresh and cooked, contains many bioactive substances with antioxidant and antiinflammatory properties and is a source of vitamins (especially C, but also E, K, B_1, B_2, B_5, B_6, B_{12}, PP), carotenoids (β-carotene, lutein, zeaxanthin), polyphenols or glucosinolates (GLS). It is also found to be rich in omega-3 fatty acids, proteins, magnesium, manganese, potassium, phosphorus and calcium. Interestingly, kale exhibit excellent calcium bioavailability (Kurilich *et al.* 1999; Podsędek 2007).

Epidemiological studies have associated diets rich in *brassica* vegetables and other GLS-containing plants with reduced risk for a number of cancers. Animal studies have

identified several GLS derivatives from *brassica* vegetables that exhibit these chemopreventive properties. A large body of literature shows that GLS derivatives modify many mammalian detoxification enzymes that make up part of our host defense against foreign chemicals, by inhibiting carcinogen activation and increasing carcinogen detoxification, resulting in clearance of carcinogens from the body (Bheemreddy *et al.* 2006; Tiwari *et al.* 2015). GLS and their decomposition products (isothiocyanates (ITCs), nitriles, thiocyanates, epithionitriles and oxazolidines) show also antioxidant, antiinflammatory, antiallergic, antifungal, antivirus, antimutagenic and antibacterial properties (Volden *et al.* 2009).

Vegetables are often eaten after cooking to consumption consistency. The quality of cooked vegetables depends on quality of the raw material as well as parameters and methods of processing. Thermal processing (especially traditional boiling) have a negative impact on the levels of vitamins, GLS and nitrates and nitrites as well as antioxidant activity in *brassica* based products. Conversely, if active myrosinase and GLS are present in the cooking water, myrosinase may hydrolase its substrate resulting in the formation of enzymatic breakdown products of GLS. However, at higher temperature, myrosinase can be inactivated by heat and GLS can be thermally degraded (Sarvan *et al.* 2014; Tiwari *et al.* 2015). The aim of this study was to examine how the process of traditional cooking in water changes the selected parameters of health quality of kale such as the content of vitamin C, β-carotene, indole-3-carbinol (I3C), indole-3-acetonitrile (I3ACN), diindolilometan (DIM), indole-3-acetic acid (I3AA), total indoles and total ITC, total polyphenols, nitrates and nitrites, as well as its antioxidant activity.

MATERIALS AND METHODS

Plant Material

The material investigated consisted of fresh kale (*B. oleracea* L. var *acephala*) leaves. The kale cultivar under investigation was *Winterbor* F_1 and was grown up at the "Krakowska Hodowla i Nasiennictwo Ogrodnicze 'Polan'" in Igołomia (Poland).

Vegetable samples were prepared for analyses directly after harvest. The leaves were first separated (5 kg green mass), then were wash under running water, next tiny cut into strips 2–3 cm in width (exterior and interior parts of the plant) and mixed to obtain the representative average laboratory samples (a minimum of three for each analysis performed on the fresh material and the same procedure was on material after cooking). Another part of fresh material was wash and then dried on the filter paper; shredded mechanically; frozen at −22C; 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 the possibly 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 min. The boiled vegetables were then prepared as described for fresh vegetables.

Analytical Methods

The content of vitamin C was determined as the sum of ascorbic acid and dehydroascorbic acid using 2,6-dichlorophenoloindophenol according to PN-A-04019:1998. Oxalic acid solution was used for extraction of the ascorbic acid.

The amount of β-carotene was measured according to the PN-90/A-75101/12 standard by extracting carotenoid from the test sample using hexane, carotenoid separation on a chromatographic column and colorimetric determination of β-carotene at a wavelength of 450 nm.

Nitrate and nitrite content according to Polish national standard using spectrophotometric method (PN 92/A-75112). The principle of the nitrates and nitrites determination was to induce a color reaction between nitrites and *N*-(1-Naphthyl)ethylenediamine dihydrochloride under acidic conditions by adding the following Griess reagents: Griess I (sulfanilamide in a defined hydrochloric acid solution) and Griess II (water solution of *N*-(1-Naphthyl)ethylenediamine dihydrochloride). Afterwards, absorbance of the formed complex was measured colorimetrically at 538 nm. Previously, nitrates were directly reduced to nitrites by pulverized cadmium.

Simultaneously, 70% methanol extracts has been prepared to determine: total polyphenols (calculated per chlorogenic acid [CGA]) – through the colorimetric measurement of colorful substances formed due to the reaction between phenolic compounds and a Folin–Ciocalteau reagent (Sigma) (Poli-Swain and Hillis 1959) and to determine antioxidant activity based on the $ABTS^{+}$ free radical scavenging ability – by a colorimetric assessment of an amount of the $ABTS^{+}$ free radical solution, which had not been reduced by the antioxidant present in the products examined (Re *et al.* 1999).

The content of total phenols in the extracts was determined spectrometrically at a wavelength of 760 nm using a RayLeigh UV-1800 spectrophotometer (Beijing Rayleigh

TABLE 1. SELECTED ANTIOXIDATIVE AND BIOACTIVE COMPUNDS, ANTIOXIDANT ACTIVITY AND NITRATES AND NITRITES OF RAW LEAVES OF KALE

Component		Mean ± SD
Vitamin C	mg/100 g f.w.	108.50 ± 3.92
β-Carotene	mg/100 g f.w.	6.12 ± 0.00
Total polyphenols	mg CGA/100 g f.w.	689.61 ± 0.22
Total isothiocyanates	μmol/g d.m.	0.21 ± 0.00
Total indoles	μmol/g d.m.	0.132 ± 0.00
Indole-3-carbinol	μmol/g d.m.	0.022 ± 0.00
Indole-3-acetic acid	μmol/g d.m.	0.07 ± 0.00
Indole-3acetonitrile	μmol/g d.m.	0.03 ± 0.00
Diindolylmethane	μmol/g d.m.	0.003 ± 0.00
Antioxidant activity	μm Trolox/g f.w.	20.50 ± 0.06
Nitrates	mg $NaNO_3$/kg f.w.	1261.54 ± 0.99
Nitrites	mg $NaNO_2$/kg f.w.	3.24 ± 0.17

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

Analytical Instrument Corporation, China according to the Folin–Ciocalteu procedure and calculated as CGA equivalents (in terms of milligrams) per 100 g of fresh or dry weight, based on a standard curve.

The method involved colorimetric determination of the amount of the colored solution of $ABTS^+$ free radical (2,2′-azinobis-(3-ethylbenzothiazoline-6-sulfonic acid) which was reduced by the antioxidants present in the test product. The absorbance was measured at a wavelength of 734 nm using a RayLeigh UV-1800 spectrophotometer. Values obtained for each sample were compared with the concentration–response curve of the standard Trolox solution and expressed as micromoles of Trolox equivalent per gram of fresh or dry weight.

To determine the content of ITC and the indolic compounds the high-performance liquid chromatography–tandem diode array–fluorescence detector system (HPLC-DAD-FLD) system (Agilent Technologies 1200 Series) equipped with a Zorbax Eclipse XDB-C8 column was applied. The content of ITC was determined by Zhang's *et al.* method (Zhang *et al.* 1996) with some modifications described by Piekarska *et al.* (2014). The indolic compounds were analyzed by method described by Piekarska *et al.* (2014) and Pilipczuk *et al.* (2015). The calibration curves used for indolic compounds quantification were generated by the integration of the areas of fluorescence peaks determined during analysis of serial dilutions of I3C (Sigma), I3ACN (Merck), I3AA (Merck) and DIM (3,3′-diindolylmethane, Sigma).

Statistical Analysis

All analyses were carried out in three parallel replications and mean ± SD were calculated for the values obtained. By the use of one-way ANOVA, the significance of differences was checked between mean values of raw and cooked material. The significance of differences was estimated with Duncan test at the critical significance level of $P \leq 0.05$. The Statistica 10.1 program was applied (StatSoft, Inc., Tulsa, OK).

RESULTS

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.

Chemical analyses confirm a high nutritional value of kale in a group of brassica vegetables. In the fresh vegetable, mean values determined for the levels of antioxidants, bioactive compounds and antioxidant activity were: 108.5 mg, for vitamin C; 6.12 mg, for β-carotene; 689.6 mg, for total polyphenols/100 g fresh weight and 20.5 μm Trolox/g fresh weight, for antioxidant activity (Table 1). Therefore, as the representative vegetable sample had been prepared for freeze-drying and the vegetables underwent such preprocessing like cleaning, cutting or grinding; the raw vegetable was also examined for the GLS breakdown products namely: I3C – 0.022, I3AA – 0.075, I3ACN – 0.032, DIM – 0.003, total indoles – 0.132 and total ITC content – 0.213 μmol/g d.m.

As a result of technological treatment, a significant fall ($P \leq 0.05$) in vitamin C, I3AA and DIM content of 78.8 and 100%, respectively, was observed and a 36.9% decrease in total polyphenols, compared with the fresh vegetable (Table 2). This process caused also a statistically significant ($P \leq 0.05$) increase in other antioxidants and bioactive compounds, i.e., β-carotene and I3C, I3ACN, total indoles and total ITC content of 11.3; 309.1; 300.0; 36.4 i 938.1%, respectively, compared with the vegetable before cooking. Simultaneously, there were no statistically significant ($P > 0.05$) changes in the antioxidant activity of kale due to cooking in comparison with the raw vegetable.

This work also investigated the levels of selected contaminants such as nitrates and nitrites, which were, respectively, 1261.5 and 3.24 mg/kg fresh weight of the vegetable (Table 1). The process of cooking contributed to statistically significant reductions in the aforementioned constituents of 20.8% in nitrates and 73.7% in nitrites compared with fresh vegetable.

DISCUSSION

Vitamin C

The examined variety of kale contained 108.5 g of vitamin C per 100 g fresh vegetable weight which is similar to the results obtained by Grajek (*ed.*) (2007) (93.0–186.0). The amount reported by Korus (2011) was similar and was 112.1 mg/100 g

TABLE 2. EFFECT OF COOKING ON SELECTED QUALITY PARAMETERS IN LEAVES OF KALE

Component		Raw (X ± SD)	Cooked (X ± SD)	Percentage of loss/increase
Vitamin C	mg/100 g d.m.	$615.30^{a} \pm 2.24$	$130.51^{b} \pm 1.08$	−78.8
β-Carotene	mg/100 g d.m.	$35.53^{b} \pm 0.01$	$39.54^{a} \pm 0.00$	+11.3
Total polyphenols	mg CGA/100 g d.m.	$4004.40^{a} \pm 1.29$	$2526.31^{b} \pm 3.62$	−36.9
Total isothiocyanates	μmol/g d.m.	$0.21^{a} \pm 0.02$	$2.18^{b} \pm 0.15$	+938.1
Total indoles	μmol/g d.m.	$0.132^{a} \pm 0.00$	$0.18^{b} \pm 0.00$	+36.4
Indole-3-carbinol	μmol/g d.m.	$0.022^{a} \pm 0.00$	$0.09^{b} \pm 0.00$	+309.0
Indole-3-acetic acid	μmol/g d.m.	$0.07^{b} \pm 0.00$	$0.00^{a} \pm 0.00$	−100.0
Indole-3-acetonitrile	μmol/g d.m.	$0.03^{a} \pm 0.00$	$0.09^{b} \pm 0.00$	+300.0
Diindolylmethane	μmol/g d.m.	$0.003^{b} \pm 0.00$	$0.00^{a} \pm 0.00$	−100.0
Antioxidant activity	μm Trolox/g d.m.	$119.01^{a} \pm 0.41$	$119.72^{a} \pm 1.19$	−0.52
Nitrates	mg $NaNO_3$/kg d.m.	$7324.25^{a} \pm 5.8$	$5801.74^{b} \pm 9.86$	−20.8
Nitrites	mg $NaNO_2$/kg d.m.	$18.90^{a} \pm 1.04$	$4.96^{b} \pm 0.08$	−73.7

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

The values in rows denoted with the same letters don't differ statistically significantly at $P \leq 0.05$.

fresh weight of the vegetable. According to Boonstra *et al.* (2002) and Davey *et al.* (2000), the content of vitamin C in kale is higher (186 mg/100 g) than this obtained in this work. However, Pfendt *et al.* (2003), as well as Sikora and Bodziarczyk (2012) noted lower levels of this component in 100 g of fresh vegetable, which were, respectively, 92.6 mg, and 52.25–77.91 mg. The differences found in the content of vitamin C in fresh vegetables may be due to such factors as, e.g., variety, stage of the yield maturity, agronomic and climatic conditions, light intensity or time of harvest (Acikgoz 2011).

Technological treatments, such as cooking, preprocessing (washing, peeling, grinding) may lead to significant falls in antioxidants, particularly in vitamin C. Such losses are also associated with leaching into the solution of this component as well as greater activity of the enzyme ascorbinase at elevated temperatures (40–70C) (Czarniecka-Skubina and Gołaszewska 2001). Ascorbic acid is susceptible to various environmental factors, such as temperature or oxygen. In addition, this vitamin is easily soluble in water; therefore, the processes conducted in aqueous medium at elevated temperature leads to larger losses (Aguero *et al.* 2005).

In this study, it has been found that vitamin C content decreased by 78.8% due to cooking, which is lower than the findings of Sikora and Bodziarczyk (2012), who noted losses in this vitamin of 89%. According to Zhang and Hamauzu (2004), heat treatment reduces vitamin C content in broccoli in the range of 19.2–65.9%. Majority of authors reported generally lower losses in vitamin C in selected brassica vegetables due to cooking: 48% in broccoli (Miglio *et al.* 2008); 46% in broccoli (Kmiecik and Budnik 1997); about 30% in cauliflower (Kmiecik and Lisiewska 1997; Cieślik *et al.* 2005). The extent of losses depends on the temperature used, length of the exposure to this temperature, the degree of grinding the product, a ratio of the vegetable's weight to the amount of water and a method of hydrothermal processing (traditional cooking, microwave cooking, etc.) (Miglio *et al.* 2008).

β-Carotene

KURILICH et al. (1999) confirms that, of all *brassica* vegetables, kale has the highest β-carotene content. The content of β-carotene determined in this work was 6119.7 μg/100 g fresh weight. The amounts found by other authors were slightly lower and were 5350 μg (Kunachowicz 2009); 5000 μg (Wartanowicz and Ziemlański 1999); 3933 μg/100 g (Sikora and Bodziarczyk 2012); and from 3800 to 4530 μg/100 g fresh weight (Korus and Kmiecik 2007). However, as Grajek (2007) claims, this value can reach even 43.80 mg/100 g that exceed significantly the results obtained in this work.

After cooking, the content of β-carotene in the examined kale increased by 11.3%. According to Gębczyński (2008) and Sikora and Bodziarczyk (2012), losses were, respectively, of 8 and 5%, whereas 45-min cooking broccoli in water resulted in β-carotene reductions of 45%, as was reported by Zhang and Hamauzu (2004). Conversely, Gliszczyńska-Świgło *et al.* (2006) and Miglio *et al.* (2008) reported that boiling and steaming of broccoli resulted in an increase in carotenoids as compared with fresh broccoli.

In some cases (e.g., in this work), heat treatment may cause an increase in carotenoids through the release of carotenoids from the carotenoid-protein complexes and changes occurring in proportions of soluble to insoluble components of vegetables (Scott and Eldridge 2005). The cooking process slightly affects changes in the content of β-carotene; however, the properties of the raw material and various parameters of the process can have an effect on levels of this component (Zhang and Hamauzu 2004).

Breakdown Products of GLS

GLS are an important group of phytochemicals and also a unique group of plant secondary metabolite compounds. GLS are unstable compounds that are on cellular disruption hydrolyzed by the myrosinase (thioglucoside glucohydrolase

EC 3.2.3.1) present in plant tissue to various bioactive breakdown products (ITCs, nitriles, thiocyanates, epithionitriles and oxazolidines) (McNaughton and Marks 2003; Girgin and El 2015). Various processes during heat treatment and subsequently (during storage or preparation of samples) may have an impact on the content of GLS in the vegetables.

Jiao *et al.* (1998) investigated the level of ITCs in *brassica* vegetables before and after cooking. Of 82 samples of *brassica* vegetables (10 different types), only three (two from kai choi and one from watercress) had ITCs after cooking. This agrees with the findings of other authors (De Vos and Blijleven 1988), who did not detect the products of the GLS breakdown in the cooked brassica vegetables. Ciska *et al.* (2009) was to investigate the effect of the boiling process on the content of ascorbigen, I3C, I3ACN and 3,3′-diindolylmethane in fermented cabbage. After 10 min of boiling, the content of free I3C and I3ACN stabilized at the level of about 80% as compared with the uncooked cabbage, but after 40 and 50 min of boiling, the total content of 3,3′-diindolylmethane in cabbage was sixfold higher than that in uncooked cabbage.

It is highly likely that in the case of kale, the preprocessing caused that the enzyme myrosinase was released to a lesser degree and only after the application of cooking the enzyme activation took place; longer heating the water and longer keeping the enzyme in its optimal temperature (i.e., until water reached 90C), activated the enzyme and resulted in the formation of breakdown products of GLS. Thus, boiling for more than a minute, or steaming for more than 4 or 5 min will lead to loss of myrosinase activity. Cooking temperature and time also affect the release of ITCs from GLS due to the inactivation of myrosinase and epithiospecifier protein (ESP) and destruction of heat-labile ITCs (Rungapamestry *et al.* 2007; Tang *et al.* 2013). It has been reported that cooking reduces ITC exposure from *brassica* vegetables by 60–90% (Shapiro *et al.* 1998; Getahun and Chung 1999). The increase in the sum of indoles and ITCs 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 1 g dry matter.

Sosińska and Obiedziński (2011) observed the same tendency in the growth of 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 these compounds increase was most likely due to the a low activity of ESP in analyzed vegetable varieties. The authors supposed also that when the myrosinase underwent dentauration during boiling of vegetables the glucobrassicin might have underwent further transformations, but nitriles could also have formed through nonenzymatic decomposition of GLS.

In the case of breakdown products of GLS, their amounts and profile depend not only on GLS-myrosinase system, but also some additional specifier protein factors. Myrosinase catalyzed degradation of GLS results in the formation of the unstable intermediate (thiohydroxamate-*O*-sulfonate) that, in the absence of specifier proteins, spontaneously becomes converted to the most desirable compounds, i.e., ITCs and indoles, exhibiting the strongest biological activity. However, the catalytic activities of these additional protein factors, which in addition to myrosinase participate in GLS degradation process, promote formation of other, less bioactive compounds. The Rungapamestry *et al.* (2006) observed the reduction of myrosinase activity during cabbage steaming or microwaving, however, this enzyme still retained some activity. They also observed parallel changes in the profile of GLS degradation products that were influenced by the type of cooking and treatment time. During cabbage steaming, the conversion of GLS to cyanoepithioalkanes decreased and the content of ITCs increased. The authors suggested that additional protein factors – ESPs – in raw and near-raw cabbage, despite possessing the highest myrosinase activity, might have blocked the rearrangement of thiohydroxamate-*O*-sulfonate to ITCs. On longer cooking, probably due to ESP denaturation, the production of ITCs was increased proportionally to the reduction in the formation of cyanoepithioalkane. Our results are consistent with these findings. Apparently, similarly to cabbage, specifier proteins are less thermally resistant than myrosinase, which explains the higher content of ITCs and indoles (spontaneously formed from indolic ITCs) in cooked kale versus raw material.

Total Polyphenols. In this study, the content of total polyphenols in kale was 689.6 mg/100 g fresh weight of the vegetable (expressed as CGA), which almost compares with the results obtained by Sikora and Bodziarczyk (2012) (676.5 mg/100 g). The levels of these compounds in the examined vegetable which were registered by other authors were lower: 384.09 mg (Korus 2011); 202.1 mg (Łata and Wińska-Krysiak 2006); 94.97 mg/100 g (Sikora *et al.* 2012) or 30–60 mg/100 g fresh weight of the vegetable (Manach *et al.* 2004).

The content of polyphenolic compounds in plant raw materials is affected by a number of factors, e.g., climatic conditions and agro-technical practices, the stage of maturity, the time of harvest, storage conditions, genetic factors, varietal diversity and the extent of damage to the vegetable tissue (Ninfali and Bacchiocca 2003). In the case of polyphenolic compounds strongly differing in terms of structure and properties, no less important are the conditions of their extraction from the raw material (e.g., both 50 and 70% methanol and 70 or 80% acetone as well as 70% acetone acidified with acetic acid, etc.), methods of analysis as well

as different manner of calculation depending on the standard used (Grajek 2007). Prior *et al.* (2005) noted, however, that a method for determining total polyphenols with Folin–Ciocalteau reagent is vitiated by an error, as this substance can react not only with polyphenols but also with numerous other compounds, thereby increasing the result obtained from the analysis of polyphenolic compounds.

Halvorsen *et al.* (2002), after examining 32 vegetables from around the world, found that kale has one of the highest content of antioxidants including polyphenols. According to these authors kale was number two, just after chilli pepper, and before other cruciferous plants such as red cabbage (third place), Brussels sprouts (eighth place) or broccoli (14th place).

In this work, cooking kale caused a 36.9%, fall in total polyphenols, which is lower than the losses found by Sikora and Bodziarczyk (2012) (56%) and those reported by Sultana *et al.* (2007) (43%). In the previous studies of Sikora *et al.* (2008), losses in total polyphenols due to hydrothermal processing were much greater, of up to 72.3%. Volden *et al.* (2009) noted that traditional cooking of cauliflower reduces the level of polyphenols by 13–37%. According Cieślik *et al.* (2005), of the various methods of processing brassica vegetables, cooking affects the reduction of polyphenols to the greatest extent (an average by 30%), particularly when the raw material previously underwent freezing (an average of 65%). Differences in the levels of phenolic compounds, found by the authors between the fresh material and the material having undergone freezing and then cooking, were the largest with regard to kale (an average of 47%). The amount of these components is strongly affected by parameters of the process as well as properties of the raw material. The losses of polyphenols due to boiling were reported also in another cruciferous vegetables, e.g., in broccoli (Zhang and Hamauzu 2004); and in kale, spinach, cabbage, swamp cabbage and shallots (Ismail *et al.* 2004). Studies by Turkmen *et al.* (2005) revealed that cooking reduces the level of polyphenols by 62%.

Antioxidant Activity. In this study, the antioxidant activity in kale was 20.5 μmol Trolox/g fresh weight of the vegetable, which is similar to the results obtained by Sikora and Bodziarczyk (2012) (22.1–47.4), Gębczyński (2008) – 18.9, Cao *et al.* (1996) – 17.7 μmol Trolox/g for $ORAC_{ROO}$ or Korus (2011) – 17.6 μmol Trolox/g fresh weight of the vegetable.

Cooking kale reduced antioxidant activity by only 0.52% compared with the raw vegetable, which is much lower than these results obtained in cauliflower by Volden *et al.* (2009) (40–58 and 19–44% for the oxygen radical absorbance capacity assay (ORAC) and the ferric reducing ability assay (FRAP) values, respectively), Pellegrini *et al.* (2004) (19.0%) and Wu *et al.* (2004) (<6%) and by Sikora and Bodziarczyk (2012), who detected a 38% decline in antioxidant activity of kale. Conversely, Cieślik *et al.* (2005) analyzing the effect of cooking on the changes in antioxidant activity of *brassica* vegetables found that this process reduced antioxidant activity by 14.9% in fresh broccoli, 15% in cauliflower, 31% in kale and by 10.8% in *Brussels* sprouts.

According to Volden *et al.* (2009), a reduction in antioxidant activity during cooking is strongly affected by a cooking method and length of the process. Water environment favors the rapid transfer of heat to the whole volume of the product. As a result, contact with heat is extended and the whole vegetable mass is heated uniformly. In such conditions antioxidants are oxidized and complexes are formed between antioxidants and other vegetable constituents, the enzymatic modifications occur as well as oxidation of antioxidants or their extraction to a solution and conversion of their active form into a prooxidant. All these reactions lead to losses in antioxidants in the vegetable, thereby reducing its antioxidant potential.

Nitrates and Nitrites

Vegetables are the major source of nitrates and nitrites in the human diet. Apart from their biological characteristics, the level of nitrates in vegetables is also affected by the type of soil, soil pH, dose and form of fertilization, time of harvest, weather conditions and other factors. The harmful effect of nitrates results from their ability to reduce nitrites. These compounds contribute to the occurrence of methemoglobinemia and are involved in the formation of carcinogenic nitrosamines (Amr and Hadidi 2001). Conversely, there is surprising hypothesis about a beneficial effect of nitrites and nitrogen oxides on the human body. Lundberg *et al.* (2006) hypothesized that the nitrate, after their bioconversion into nitrite or as *N*-nitroso compounds, are the main factor responsible for the beneficial role of vegetables in the prevention of cardiac diseases.

The kale variety analyzed in this study contained 1261.5 mg nitrates and 3.24 mg nitrites per kilogram fresh weight of the vegetable and these values are similar to the values 1324.83/3.52 mg noted by Sikora and Bodziarczyk (2012) and 248-2810 mg reported by Korus and Lisiewska (2009), but only with regard to nitrates; in the case of nitrite, the author reported lower values (0.14–0.95 mg). The content of nitrites determined in *brassica* vegetables by Śmiechowska (2002) ranged within 0.2–3.3 mg/kg, which is consistent with our results.

Due to toxic effects of nitrates, the Commission Regulation (EC) No 1881/2006 of 19 December 2006 (currently in force in Poland) sets maximum levels for certain contaminants in foodstuffs to ensure the safety of the consumer health. However, this regulation provides maximum acceptable levels of nitrates only for lettuce and frozen spinach.

In this study, cooking resulted in decreases in the content of nitrates and nitrites of 20.8 and 73.7%, respectively. Sikora and Bodziarczyk (2012) reported a larger 78% fall in the nitrates and a smaller 67% decrease in nitrites compared with the level determined in this work. According to Shimada and Ko (2004), the losses in nitrates due to cooking range broadly from 14 to 79%, which agrees with our findings. Leszczyńska *et al.* (2009) found that cooking green and white cauliflower resulted in losses in nitrates of 38 and 72% and in nitrites of 7 and 10%, respectively.

CONCLUSIONS

In conclusion, 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 significant content of bioactive substances, high antioxidant activity and there were no objections with regard to the level of nitrates and nitrites.

Hydrothermal treatment (cooking) resulted in a statistically significant increase in the level of β-carotene, I3C, I3ACN, total indoles and total ITC, and a substantial reduction in the content of vitamin C, polyphenols, DIM, I3AA and nitrate and nitrites, compared with the raw vegetable. As regards antioxidant activity, there were no statistically significant changes.

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