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Effect of preliminary processing and method of preservation on the content of selected antioxidative compounds in kale (*Brassica oleracea* L. var. *acephala*) leaves

Anna Korus *, Zofia Lisiewska

Department of Raw Materials and Processing of Fruit and Vegetables, Agricultural University of Krakow, 122 Balicka Street, 30-149 Krakow, Poland

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ABSTRACT

Vitamin C and polyphenol content as well as total antioxidative activity were investigated in fresh leaves of kale; in leaves after blanching or cooking; and in frozen and canned leaves. In 100 g fresh matter, kale leaves contained 384.9 mg polyphenols and 112.1 mg vitamin C, with a Trolox equivalent antioxidant capacity (TEAC) level of 1175 μM Trolox. Of the polyphenols identified in kale leaves, ferulic acid occurred in the highest amount (240.44 mg/100 g, constituting 62% of total polyphenols). Freezing was a better method of preserving kale leaves since the loss of nutritive constituents was lower than in the case of canning. Depending on preliminary processing and storage temperature, after one-year storage frozen leaves contained 82.9–171.3 mg polyphenols and 39.3–65.4 mg vitamin C, with TEAC at the level of 501–681 μM Trolox in 100 g. In canned leaves these values were: 91.3–94.1 mg polyphenols, 16.1–19.3 mg vitamin C and 268–293 μM Trolox.

1. Introduction

Diet can play an important role either in precipitating or preventing diseases. Vegetables, and *Brassicas* in particular, are an important component of the European diets, they are also some of the most consumed vegetables in the world (Lin & Harnly, 2009). Kale is a leafy green vegetable belonging to the *Brassicaceae* family, a group of vegetables including cabbage, broccoli, cauliflower, and brussel sprouts, with a high content of health-promoting phytochemicals. Kale has a high concentration of vitamins, minerals, dietary fibre, and antioxidative compounds (Podsędek, 2007). The concentration of total polyphenols and flavonoids determined in kale (1039 mg GAE/kg fw) exceeded that found in all other vegetables; it was higher than that in onion, green bean, broccoli or leek (Dragović-Uzelac et al., 2009; Hertog, Hollman, & Katan, 1992). Kale is also a good source of vitamin C (107 mg/100 g fw) and carotenoids (2.7 mg/100 g fw) (Sikora, Cieślik, Leszczyńska, Filipiak-Florkiewicz, & Pisulewski, 2008). Cao, Sofic, and Prior (1996) reported that the antioxidant score decreased in the following order: kale > garlic > spinach > brussel sprouts > broccoli flowers. Record, Dreosti, and McInerney (2001) have suggested that the phytochemical content and corresponding antioxidant activity of vegetables contribute to their protective effects against chronic and degenerative diseases.

Vegetables contain many antioxidant compounds. Polyphenols constitute the largest group of these biologically active constituents. Vitamin C and polyphenols are commonly recognised as a major naturally occurring nutrients and antioxidants in diet. Epidemiological studies have shown a correlation between an increased consumption of phenolic antioxidants and a reduced risk of cardiovascular disease and certain types of cancer. Processing and preparation, especially thermal treatment, which are applied prior to consumption, may affect these phytochemicals. Vitamin C is one of the most sensitive vitamin. For this reason it is often used to evaluate the influences of food processing on vitamin contents (Bognár, 1989). Like vitamin C, phenolic antioxidants are water-soluble and can be leached from vegetables tissues by processing in water (Gil, Ferreres, & Tomas-Barberan, 1999).

Many people have no opportunity to eat fresh vegetables every day and frequently use frozen vegetables, mainly for convenience, time-saving and practical reasons (Ninfali & Bacchiocca, 2003). Food freezing is among the most efficient and adequate preservation methods. The low temperatures commonly used for frozen foods can maintain initial quality and nutritive value practically unchanged. Canning is one of the main methods used by the food industry to preserve seasonally available vegetables. As ready-to-eat products, canned vegetables are easily prepared for consumption and can be stored for long periods at above-zero temperatures. However, Jaworska, Kmiecik, and Maciejaszek (2001) pointed out that the nutritive value of

* Corresponding author. Tel./fax: +48 12 662 47 57.
E-mail address: a.korus@ur.krakow.pl (A. Korus).

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canned vegetables is significantly reduced, and Hunter and Fletcher (2002) found that the antioxidative activity in sterilized vegetables is lower than in frozen products.

The aim of the present work was to investigate qualitative changes in kale leaves after preliminary processing and preservation by freezing and canning. The level of selected antioxidative constituents, namely vitamin C and polyphenol compounds, formed the criterion for evaluation. The investigation covered raw kale leaves, leaves after preliminary processing (blanching and cooking), two kinds of frozen products; one obtained using the traditional method (kale leaves blanched before freezing) and the other a convenience type food product (kale leaves cooked to consumption consistency before freezing); and two kinds of canned products: from cut and from ground leaves. Both frozen and canned products were evaluated directly after processing and after one year of storage.

2. Materials and methods

2.1. Materials

The material investigated consisted of fresh kale (*Brassica oleracea* L. var *acephala*) leaves, leaves after blanching, leaves after cooking to consumption consistency, frozen products stored at a temperature of −20 °C and −30 °C, and sterilized canned products. The various products were evaluated directly after processing (0 months) and after 12 months of storage. The kale cultivar under investigation was Winterbor F_1, produced by the Dutch firm Bejo Zaden b.v. (Netherlands). Its leaves are dark green and strongly corrugated. This cultivar is classed among those most resistant to disease, yellowing of lower leaves and frost damage. Kale was grown from seedlings planted in an experimental field located in southern Poland, on the western outskirts of Krakow (50°04′N, 19°51′W). Seedlings with 3–4 leaves were planted at spacings of 50 × 50 cm in late June. Mineral fertilization was applied according to the fertility of soil and the nutritional requirements of the crop.

A single harvest was carried out 14 weeks after planting, during the last five days of September. Entire plants were cut and damaged leaves discarded. The main veins were then removed from the usable leaves (those with good colour and undamaged by disease or pests). From the fresh leaves thus prepared, a batch of 5 kg was randomly sampled for chemical analysis of the raw material. The remaining material was blanched or cooked to obtain frozen or canned products.

2.2. Preparation of frozen kale

The freezing of kale leaves was preceded by technological procedures: washing, cutting into strips 2–3 cm in width, blanching or cooking, and cooling and dripping on sieves. Two types of frozen product were obtained: the traditional method (variant I) from blanched kale leaves (FB) and the modified method (variant II) from kale leaves cooked to consumption consistency (FC) to obtain a ready-to-eat product which, when defrosted, requires only heating and seasoning by the consumer.

In variant I, kale was blanched in a stainless steel vessel for 2.5 min at 96–98 °C, the proportion of water to the raw material being 5:1 by weight. The blanching was regulated so as to reduce the activity of catalase and peroxidase to a level not exceeding 5% of the initial activity. After blanching, the material was cooled in cold water and left to drip on sieves for 30 min. In variant II, kale was cooked in a stainless steel vessel in water for 10 min at a temperature of about 100 °C, the proportion of water to the material being 5:1 by weight. After cooking, the material was left of sieves and cooled in a stream of cold air.

The material from blanched and cooked samples was packed in 500 g polyethylene bags and frozen at −40 °C in a blast freezer (Feutron GmbH, model number 3626-51). One part was frozen to −20 °C for 90 min and the other to −30 °C for 120 min. Frozen products were placed in a chamber freezer at −20 °C and −30 °C until evaluation.

2.3. Preparation of canned kale

The canning of vegetables using the sterilization method was preceded by the following technological procedures: washing; blanching; cutting into strips 2–3 cm wide or grinding; filling metal cans 0.8 l in volume; pouring on hot water; closing and preserving (sterilization). Two kinds of products were obtained: from cut (CC) and ground (CG) raw material. Ground kale can be used as an ingredient in vegetable mixtures or as a side dish. Blanching was carried out as in the case of freezing. Leaves were ground in an electric mill of 8 mm mesh size. No salted brine was used in the production of canned kale since earlier tests showed that kale processed without salt had better flavour. Sterilization was carried out in a pressure sterilizer using the following stages of thermal processing: increasing the temperature to 100 °C–10 min; increasing the temperature from 100 °C to 118 °C–15 min; sterilization at 118–120 °C–80 min; cooling to 100 °C–15 min; cooling to 30 °C–10 min. When completely cool and dry, cans containing processed kale were placed in a cool chamber at a temperature below 10 °C and stored until analysis.

2.4. Chemical determination

The vitamin C content was determined as the sum of ascorbic acid (AA) and dehydroascorbic acid (DHAA) using the spectrophotometrical method (ISO, 1984). Oxalic acid solution (2%) was used for extraction of the ascorbic acid. After quantitative reduction of 2,6-dichlorophenolindophenol dyestuff by ascorbic acid, and extraction of the excess dyestuff using xylene, the excess was measured at 500 nm in a Shimadzu UV 160A spectrophotometer and compared with vitamin C as the reference standard.

Phenolic compounds were determined by high-performance liquid chromatography (HPLC) using a liquid chromatograph equipped with a Merck-Hitachi L-7455 diode array detector (DAD). The 0.5 g of sample was mixed with the citrate buffer solution at pH 5.5, and then specific enzymes were added (Drum pectinase 263 from Seclin, France; β-glucosidase, hesperidinase and sulphatase type H-2 from Sigma). The whole was incubated for 1 h in a water bath (40 °C) and then left for 20 h in the dark chamber for enzymatic hydrolysis. After that, 5 ml of pure methanol were added to the samples which were then placed for 10 min in an ultrasonic bath. After centrifuging in a laboratory centrifuge, the samples were subjected to HPLC analysis. Phenolic compounds were estimated using a LiChroCART® 125-3 Purospher® RP-18 (5 μm) column (Merck) thermostated at a temperature of 30 °C. The separation was carried out using an 80% solution of acetonitrile in 4.5% formic acid (reagent A), and 2.5% acetic acid (reagent B), the flow rate was 1 ml/min. The reagents were varied according to the following program: linear increase in the concentration of reagent A from 0% to 15% between minute 0 and 7, and then to 2% between minute 8 and 15, while decreasing the concentration of reagent B to 80%, followed by an increase in A to 100% and a decrease in B to 0% from minute 16 up to the end of the analysis. After eluting the column in the latter conditions (A = 100%, B = 0%), the A concentration was decreased to 0% in order to stabilize the column for 10 min before the use of the next sample. During the analysis, the solutions were degassed in an ultrasonic bath (Merck). The runs were moni-

tored at the following wavelengths (λ): phenolic acids – 320 nm, flavones – 340 nm, flavonols – 360 nm, anthocyanins – 520 nm. The compounds were identified on the basis of the spectra in the range of 200–600 nm and the retention times compared to standards. Recoveries from the samples were measured by spiking pure standards of the identified polyphenols into the extraction solution. The determinations were carried out in three replications. Recoveries of the standards from kale samples were in range 85–90%.

Antioxidant activity (TEAC) was evaluated by means of spectrophotometric method as well as the $ABTS^{\cdot+}$ (2, 2′-azinobis-(3-ethylbenzothiazoline-6-sulfonic acid) (Re et al., 1999). The kale leaves used in the investigation were homogenized in a laboratory homogenizer. One gram of the homogenized sample was extracted with 25 cm^3 HCl (0.16 mol/dm^3) in 80% methanol at boiling temperature for 30 min under a reflux condenser. The extract was then cooled and rotated for 20 min (4000 g), and stored at −20 °C. An aqueous solution of $ABTS^{\cdot+}$ (2,2′-azino-bis(3-ethylbenzotialozline-6-sulfonic acid), Sigma) of 7 mmol/dm^3 was mixed with aqueous solution of potassium persulfate (2.45 mmol/dm^3) (both heated to 30 ± 0.5 °C) and left for the night to produce the $ABTS^{\cdot+}$ cation-radical. The $ABTS^{\cdot+}$ solution was diluted with the PBS buffer (phosphate buffered saline, Sigma) to obtain a working solution of 1.05 ± 0.05 absorbance in such a way that the initial absorbance of the test solution with the extract added would be 0.7. Then, 2 cm^3 of the working solution of $ABTS^{\cdot+}$ was mixed either with 1 cm^3 of PBS (blank) or with the extract appropriately diluted with PBS (so that the radical-scavenging degree would not exceed 60%). Next, 10 min after the reaction had been initiated, an absorbance was measured at wavelengths 734 nm, using Shimadzu UV-160A spectrophotometer. The results were expressed as the Trolox equivalent antioxidant activity (TEAC) in μM of Trolox/100 g of fresh matter. Trolox was prepared in 2.5 mM phosphate buffer saline, pH 7.4 (PBS).

2.5. Statistical analysis

Statistical analysis allowing a comparison of the content of antioxidant compounds was carried out using single-factor analysis of variance (ANOVA) on the basis of the Snedecor F and Student's t tests, and the least significant difference (LSD) was calculated at the probability level $p < 0.01$. The Stastica 6.1 program was used.

3. Results and discussion

3.1. Effect of blanching and cooking kale leaves on the level of vitamin C, polyphenol constituents and antioxidative activity

Fresh kale leaves contained 112.1 mg vitamin C and 384.9 mg total identified polyphenols, with antioxidative activity of 1175 μM Trolox/100 g fresh matter (Table 1). Of the polyphenols identified in kale leaves, ferulic acid occurred in the highest amount (240.44 mg/100 g, constituting 62% of total polyphenols). The content of kaempferol (59.64 mg/100 g) and caffeic acid (41.54 mg/100 g) was considerably lower, comprising 15% and 11% of polyphenols respectively. The content of quercetin, *p*-coumaric acid and sinapinic acid constituted less than 5% of total polyphenols.

The content and stability of phyto-chemical compounds in food after processing is essential in evaluating the nutritional value of vegetables rich in these constituents. Thermally processed vegetables have for a long time been considered to have a lower nutritional value than fresh produce. This is based on the fact that vitamin C and other thermo-labile compounds may lose their activity due to oxidation or in consequence of leaching into the water during home cooking or industrial processing, such as blanching (Roy, Juneja, Isobe, & Tsushida, 2009). Blanching is an indispensable treatment preceding the freezing of most vegetables; it ensures the retention of natural taste and smell, inactivates native enzymes and stabilizes a number of constituents of the raw material. Blanching is also essential in the production of sterilized canned products in airtight containers. In kale leaves blanching brought about a 34% decrease in the content of vitamin C, a 51% decrease in polyphenols and a 33% decrease in antioxidative activity compared with the raw material. The loss of polyphenol constituents was also high; the lowest loss was in caffeic acid (28%) and the highest (55%) in ferulic acid. Considerable losses in vitamin C content and decreases in antioxidative activity in vegetables after blanching have been reported by numerous authors. Selman (1994) showed that blanching leaf vegetables brought about losses of 20–70% in vitamin C, while Puupponen-Pimiä et al. (2003) reported losses of 20–30% in *brassicas*. When testing various methods of blanching leaf vegetables, Ponne, Baysal, and Yuksel (1994) also found losses of vitamin C of up to 65% after traditional blanching in water. Amin, Norazaidah, & Emmy Hainida, 2006 reported decreases of up to 51% in the level of polyphenols and 56% in antioxidative activity in blanched spinach. This may have been due to the blanching time and temperature, which could have reduced the antioxidant compounds and antioxidant activity. On the other hand, Oboh (2005) found an increase in the level of polyphenols in blanched green leaf vegetables, attributing it to the possible decomposition of tannins in the vegetables to simple phenols.

Compared with the raw material, the levels of vitamin C, polyphenols and antioxidative activity in cooked kale leaves were reduced by 57%, 73% and 45% respectively. The losses of different polyphenol constituents were also significant, ranging from 64% for caffeic acid to 82% for *p*-coumaric acid (Table 1). The boiling process at above 95 °C has been shown to decompose the antioxidant components of vegetables (Hunter & Fletcher, 2002). Amin et al. (2006) reported that cooking had a reducing effect on the

Table 1
Antioxidant compounds and antioxidant activity (TEAC) of raw, blanched and cooked kale.[a]

Compound (mg/100 g)	Raw	Blanched	Cooked	LSD $p < 0.01$
Phenolic compounds, quercetin	14.30 ± 0.07	7.70 ± 0.24 (46)[b]	4.74 ± 0.15 (67)	0.384
Kaempferol	59.64 ± 1.62	30.51 ± 1.10 (49)	18.36 ± 0.52 (69)	2.689
Caffeic acid	41.54 ± 0.78	30.06 ± 0.69 (28)	14.84 ± 4.35 (64)	5.935
p-Cumaric acid	11.35 ± 0.84	6.74 ± 0.14 (41)	2.08 ± 0.07 (82)	1.132
Sinapic acid	17.62 ± 0.24	11.89 ± 0.95 (33)	5.72 ± 0.67 (68)	1.156
Ferulic acid	240.44 ± 3.63	107.67 ± 1.84 (55)	58.32 ± 1.81 (76)	5.911
Total phenolic compounds[c]	384.9 ± 0.7	194.6 ± 3.0 (49)	104.1 ± 2.0 (73)	9.084
Vitamin C	112.1 ± 48.9	74.5 ± 3.0 (34)	48.2 ± 3.0 (57)	25.52
TEAC, μM Trolox/100 g	1175 ± 22	792 ± 22 (33)	641 ± 31 (45)	44.5

[a] Values are presented as mean value ± SD ($n = 3$) and expressed in fresh matter.
[b] Values in brackets represent the percentage loss compared to raw kale.
[c] Total content of all individual compounds combined.

Table 2
Phenolic compounds of processed kale.[a]

Type of product	Phenolic compounds (mg/100 g)						
	Quercetin	Kaempferol	Caffeic acid	*p*-Cumaric acid	Sinapic acid	Ferulic acid	Total phenolic compounds[b]
0 months stored							
Frozen kale							
FB, −20 °C	7.23 ± 0.29 (49)[c]	28.31 ± 0.89 (53)	28.13 ± 0.35 (32)	5.36 ± 0.75 (53)	11.63 ± 1.08 (34)	107.54 ± 4.29 (55)	188.2 ± 6.9 (51)
FB, −30 °C	7.36 ± 0.46 (49)	28.87 ± 1.73 (52)	28.95 ± 1.23 (30)	5.58 ± 0.37 (51)	11.82 ± 0.52 (33)	107.03 ± 6.66 (55)	189.6 ± 4.7 (51)
FC, −20 °C	4.66 ± 0.42 (67)	17.37 ± 0.40 (71)	14.58 ± 0.19 (65)	1.58 ± 0.11 (86)	5.26 ± 0.45 (68)	56.57 ± 5.09 (76)	100.0 ± 5.5 (74)
FC, −30 °C	4.69 ± 0.32 (67)	18.08 ± 0.84 (70)	14.63 ± 1.10 (65)	1.55 ± 0.18 (86)	5.62 ± 0.40 (68)	56.84 ± 3.50 (76)	101.4 ± 3.8 (74)
LSD $p < 0.01$	0.789	2.242	1.773	0.894	1.398	10.465	11.33
Canned kale							
CC	2.33 ± 0.09 (84)	11.55 ± 0.12 (81)	11.73 ± 0.13 (72)	2.74 ± 0.18 (76)	4.48 ± 0.11 (75)	72.80 ± 3.14 (70)	95.6 ± 3.22 (75)
CG	2.70 ± 0.09 (81)	12.84 ± 0.39 (78)	12.20 ± 0.23 (71)	2.84 ± 0.22 (75)	4.44 ± 0.22 (75)	74.67 ± 2.90 (69)	99.7 ± 2.99 (74)
LSD $p < 0.01$	0.170	0.389	0.353	n.s.[d]	n.s.	n.s.	n.s.
After 12 months stored							
Frozen kale							
FB, −20 °C	6.23 ± 0.81 (56)	26.88 ± 2.13 (55)	25.59 ± 0.81(38)	5.16 ± 0.32 (55)	10.53 ± 1.04 (40)	88.30 ± 5.84 (63)	162.7 ± 6.6 (58)
FB, −30 °C	6.64 ± 0.18 (54)	27.90 ± 1.35 (53)	26.72 ± 1.35 (36)	5.49 ± 0.22 (52)	11.12 ± 0.33 (37)	93.42 ± 4.99 (61)	171.3 ± 7.2 (55)
FC, −20 °C	4.24 ± 0.45 (70)	15.77 ± 0.78 (74)	14.14 ± 0.72 (66)	1.48 ± 0.04 (87)	5.06 ± 0.37 (71)	42.18 ± 1.75 (82)	82.9 ± 2.2 (78)
FC, −30 °C	4.32 ± 0.09 (70)	16.92 ± 1.88 (72)	14.57 ± 0.36 (65)	1.50 ± 0.03 (87)	5.41 ± 0.26 (69)	54.88 ± 3.47 (77)	97.6 ± 3.6 (75)
LSD $p < 0.01$	0.985	3.382	1.842	0.409	1.235	12.720	10.91
Canned kale							
CC	2.24 ± 0.12 (84)	10.90 ± 0.07 (82)	11.19 ± 0.14 (73)	2.57 ± 0.15 (77)	4.05 ± 0.18 (77)	70.39 ± 1.53 (71)	91.3 ± 1.65 (76)
CG	2.42 ± 0.09 (83)	11.43 ± 0.39 (81)	11.78 ± 0.23 (72)	2.59 ± 0.22 (77)	4.32 ± 0.22 (75)	71.54 ± 2.90 (70)	94.1 ± 2.99 (76)
LSD $p < 0.01$	n.s.	0.693	0.464	n.s.	n.s.	n.s.	n.s.
Total LSD $p < 0.01$	0.678	2.172	1.372	0.562	1.008	9.176	8.92

[a] Values are presented as mean value ± SD (n = 3) and expressed in fresh matter.
[b] Total content of all individual compounds combined.
[c] Values in brackets represent the percentage loss compared to raw kale.
[d] n.s. - not significant.

antioxidative components of leaf vegetables. Gil et al. (1999) showed that boiling fresh-cut spinach for 10 min released 50–60% of its antioxidant compounds into the cooking water. When cooking vegetables, the loss of vitamin C may be as high as 75%. Petersen (1993) attributes these losses to the leaching of water-soluble vitamins into water and to the destructive effect of oxygen. According to Sikora et al. (2008), the degree of polyphenol degradation depends to a great degree on processing time and the size of vegetables. Ewald, Fjelkner-Modig, Johnsson, Sjoholm, and Akesson (1999) reported that boiling, microwaving, frying or further warm holding did not affect the level of polyphenols, quercetin and kaempferol in onions, green beans and peas. On the other hand, losses of polyphenols due to boiling or blanching were reported in selected cruciferous vegetables (Sikora et al., 2008); broccoli (Zhang & Hamauzu, 2004); and in kale, spinach, cabbage, swamp cabbage and shallots (Ismail, Zamaliah, & Chin, 2004), probably due to the dissolution of polyphenols into the cooking water. Price, Casuscelli, Colquhoun, and Rhodes (1998) reported that after cooking for 15 min, only 18% of phenolic compounds in broccoli were retained in the cooked tissue, the remainder being largely leached into the cooking water.

The various effects of cooking on antioxidant capacity can be observed in different vegetables. In some vegetables, boiling can contribute to the suppression of oxidation by antioxidants due to the thermal inactivation of oxidative enzymes (Yamaguchi et al., 2001). In addition, the boiling process may destroy the cell walls and sub-cellular compartments, releasing potent radical-scavenging antioxidants. Zhang and Hamauzu (2004) recorded decreases in the level of the antioxidative activity in cooked broccoli and Ismail et al. (2004) in cooked kale, spinach and cabbage. In contrast, Turkmen, Sari, and Velioglu (2005) reported that boiling, microwave cooking and steaming brought about significant increases in the total antioxidative activity of pepper, green beans, broccoli and spinach. Gahler, Otto, and Bohm (2003) reported an improvement in the antioxidative activity of tomatoes after heat treatment due to the increased release of phyto-chemicals from the matrix. Increases in the level of antioxidative activity in broccoli and cauliflower during boiling were reported by Wachtel-Galor, Wong, & Benzie, 2008. This effect is perhaps due to the production of redox-active secondary plant metabolites or breakdown products. Changes in the level of different chemical constituents of vegetables subjected to such preliminary treatments in water as blanching or cooking may result from the degradation or leaching of constituents into the water and also from absorption or release of water by the product. The extent of these changes depends on the time and temperature of thermal processing and also on the ratio of the amounts of the medium to the weight of vegetables (Amin et al., 2006).

3.2. Effects of preservation methods on antioxidant compounds levels and antioxidant activity

Most vegetables are seasonal crops and in order to ensure their availability all the year round, various preservation methods have to be used. Canning and freezing are the most frequently used techniques in vegetable processing. Freezing is a highly efficient method of keeping vegetables for longer storage periods with the original characteristics mostly undisturbed.

The process of freezing kale leaves did not significantly reduce the level of analysed antioxidants or their antioxidative activity (Tables 2 and 3). Compared with the material after preliminary processing (blanching, cooking), kale leaves directly after freezing showed average decreases of 3% in total polyphenols, 2% in vitamin C and 7% in TEAC. However, differences in the level of antioxidants between the products depended on the methods of preliminary processing of the raw material before freezing. In frozen products from blanched raw material (FB), the mean content of total polyphenols and vitamin C was 189.5 mg and 72.8 mg respectively, and TEAC was 739 μM Trolox/100 g; in products from cooked raw material (FC), the levels were 47%, 34% and 19% lower respectively.

Table 3
Vitamin C and antioxidant activity (TEAC) of processed kale.[a]

Type of product	Vitamin C (mg/100 g)	TEAC (μM Trolox/100 g)
0 months stored		
Frozen kale		
FB, −20 °C	72.3 ± 2.3 (36)[b]	733 ± 111 (38)
FB, −30 °C	73.3 ± 2.6 (35)	744 ± 131 (37)
FC, −20 °C	47.6 ± 2.5 (58)	591 ± 82 (50)
FC, −30 °C	47.9 ± 2.5 (57)	600 ± 70 (49)
LSD $p < 0.01$	5.19	140.1
Canned kale		
CC	33.8 ± 2.9 (70)	394 ± 63 (66)
CG	25.1 ± 2.0 (78)	344 ± 67 (71)
LSD $p < 0.01$	6.24	n.s.
After 12 months stored		
Frozen kale		
FB, −20 °C	58.6 ± 2.1 (48)	622 ± 150 (47)
FB, −30 °C	65.4 ± 2.4 (42)	681 ± 191 (42)
FC, −20 °C	39.3 ± 1.7 (65)	501 ± 82 (57)
FC, −30 °C	43.5 ± 2.0 (61)	551 ± 101 (47)
LSD $p < 0.01$	4.28	n.s.
Canned kale		
CC	19.3 ± 0.8 (83)	293 ± 58 (75)
CG	16.1 ± 0.9 (86)	268 ± 49 (77)
LSD $p < 0.01$	2.07	n.s.
Total LSD $p < 0.01$	3.69	213.4

[a] Values are presented as mean value ± SD ($n = 3$) and expressed in fresh matter.
[b] Values in brackets represent the percentage loss compared to raw kale.

Compared with FC, the FB samples also contained more polyphenol constituents, ranging from 56% (quercetin) to 248% (*p*-coumaric acid) on average (Table 2).

Roy et al. (2009) postulate that the effect of thermal processing on the level of polyphenols and antioxidative activity depends on the type of product. Hunter and Fletcher (2002) showed that the antioxidative activity of sterilized vegetables was lower than that of fresh or frozen material. Podsędek (2007) also suggested that the level of antioxidants in vegetables after thermal processing is lower than in the raw material. This is probably attributable to the degradation of bioactive compounds and absorption of water during cooking, resulting in dilution of the active compounds. Furthermore, the high temperatures involved cause considerable losses of thermo-labile constituents in sterilized products. The most common adverse changes which occur during technological processing and lead to decreases in the antioxidative activity of the raw material are oxygenation, the formation of antioxidant complexes with other constituents in food, enzymatic modifications, and the transformation of the active form of an antioxidant into the inactive one. It should be stressed that the degradation of and changes in antioxidative compounds not only depend on the type of technological treatment but also on its duration, the temperature applied and the degree of comminution of the raw material.

The results of the present work show that sterilization of kale leaves brought about a marked decrease in the content of total polyphenols (50% on average), accompanied by losses in individual polyphenol constituents, ranging from 32% (ferulic acid) to 67% (quercetin) (Table 2). The losses of vitamin C (60%) and antioxidative activity (53%) were even greater (Table 3). It was also found that the degree of comminution of the leaves affected only vitamin C content. Canned products (CC) contained 35% more vitamin C than CG products.

3.3. Effects of storage on antioxidant compounds levels and antioxidant activity

After one year of storage, the total content of polyphenols in frozen kale was on average 12% lower than that found in frozen products directly after freezing (Table 2). In FB frozen products the mean content of polyphenol compounds was 12% higher than in MG; in 100 g fresh matter, the former contained 90.86 mg ferulic acid; 27.3 mg kaempferol; 26.16 mg caffeic acid; 10.83 mg sinapinic acid; 6.44 mg quercetin; and 5.33 mg *p*-coumaric acid on average. Storage temperature was not observed to have a significant effect on the level of the polyphenol constituents analysed in any of the investigated frozen products.

If frozen foods are handled and processed properly, their nutritive value can be retained during storage. If enzymes that accelerate the oxidation of vitamin C are inactivated by blanching, freezing causes negligible losses of vitamin C in vegetables. Blanching as the pretreatment ensures good preservation of frozen products and very good stability of the vitamin (Leskova et al., 2006). Jaworska and Kmiecik (2000) supported by the present study, the degree of vitamin C decomposition during refrigerated storage depends not only on the temperature or the period of storage but also on the blanching or cooking previously carried out, when considerable losses of this vitamin occur. Compared with leaves directly after freezing, frozen kale leaves after one year of storage contained 13% (FC) or 16% (FB) less vitamin C (Table 3). Products from blanched raw material contained 49% more vitamin C than those from cooked raw material. In both kinds of frozen leaves, storage at −30 °C brought about lower decreases in vitamin C. A similar correlation between lower storage temperatures and better retention of vitamin C was reported by Gębczyński and Lisiewska (2006) in frozen broccoli.

As in the case of polyphenols and vitamin C, a mean 12% decrease in TEAC was also recorded after one year of storage. In MB frozen products it was an average of 652 μM Trolox/100 g and 19% lower in MG. Storing frozen vegetables at the lower temperature of −30 °C resulted in 10% (average) better maintenance of activity than storage at −20 °C. Many authors have pointed out that antioxidative activity in frozen vegetables decreases during storage (Gębczyński & Lisiewska, 2006; Hunter & Fletcher, 2002).

One-year storage of canned products increased losses in vitamin C and TEAC of 40% and 24% respectively compared with products directly after sterilization (Table 3). Considerable losses of vitamin C after 12-month storage were also reported in canned spinach by Jaworska et al., 2001. The authors explain this finding by the sensitivity of vitamin C to high temperature and the long period of storage.

However, losses of individual polyphenol constituents were lower in the canned products evaluated (Table 2). After one year of storage, the constituent with the highest content was ferulic acid: 70.39 mg (CC)–71.54 mg (CG) in 100 g fresh matter; that with the lowest content was quercetin: 2.24 mg (CC)–2.42 mg (CG). Blanching, processing and one-year storage of kale leaves resulted in total losses of 85% in vitamin C, 76% in total phenolic compounds and 76% in TEAC (Tables 2 and 3). The results confirm that canning produces considerably higher losses of bioactive constituents in plant raw materials than freezing. Total losses in frozen kale leaves due to blanching or cooking, freezing and one-year storage were 54% for vitamin C, 67% for total phenolic compounds and 48% for TEAC (Tables 2 and 3).

4. Conclusions

Modern societies expect food products to be not only convenient but also healthy and nutritious. Freezing can be regarded as a safe method of preserving vegetables since no chemical preservatives are used in the process. Freezing is among the most frequently used methods of preserving plant raw materials both in the food industry and in households. The advantage of canned vegetables, however, is that the final product can be used without any preparation other than being briefly heated. The drawback

undoubtedly lies in the considerable losses of nutritive constituents which pass into the brine and the loss of thermo-labile vitamins during high temperature thermal processing. The results of this paper show that freezing is a better method of preserving kale leaves since the loss of nutritive constituents is lower than in the case of canning. After 12 months of storage, frozen products retained 22–45% polyphenols, 35–58% vitamin C and 43–56% of the initial TEAC level depending on the type of preliminary processing and storage temperature, while canned products retained 24%, 14–17% and 23–25% respectively depending on the degree of comminution of the raw material. Frozen products from blanched raw material contained the highest amounts of antioxidants; total polyphenols, vitamin C and TEAC were 85%, 49% and 24% respectively higher than in frozen products obtained from cooked raw material, and 80%, 247% and 132% higher than in canned products.

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References

Amin, I., Norazaidah, Y., & Emmy Hainida, K. I. (2006). Antioxidant activity and phenolic content of raw and blanched *Amaranthus* species. *Food Chemistry, 94*, 47–52.

Bognár, A. (1989). Untersuchungen über den Einfluss der Temperatur- und Verpackung auf den Genuss- und Nährwert von frischem Gemüse und Obst bei der Lagerung im Kühlschrank. *Ernährungs Umschau, 36*, 254–263.

Cao, G., Sofic, E., & Prior, R. L. (1996). Antioxidant capacity of tea and common vegetables. *Journal of Agricultural and Food Chemistry, 44*, 3426–3431.

Dragović-Uzelac, V., Kovačević, D. B., Levaj, B., Pedisić, S., Mezak, M., & Tomljenović, A. (2009). Polyphenols and antioxidant capacity in fruits and vegetables common in the Croatian diet. *Agriculturae Conspectus Scientificus, 74*, 175–179.

Ewald, C., Fjelkner-Modig, S., Johnsson, K., Sjoholm, I., & Akesson, B. (1999). Effect of processing on major flavonoids in processed onions, green beans and peas. *Food Chemistry, 64*, 231–235.

Gahler, S., Otto, K., & Bohm, V. (2003). Alterations of vitamin C, total phenolics, and antioxidant capacity as affected by processing tomatoes to different products. *Journal of Agricultural and Food Chemistry, 51*, 7962–7968.

Gębczyński, P., & Lisiewska, Z. (2006). Comparison of the level of selected antioxidative compounds in frozen broccoli produced using traditional and modified methods. *Innovative Food Science and Emerging Technologies, 7*, 239–245.

Gil, M. I., Ferreres, E., & Tomas-Barberan, F. A. (1999). Effect of postharvest storage and processing on the antioxidant constituents (flavonoids and vitamin C) of fresh cut spinach. *Journal of Agricultural and Food Chemistry, 47*, 2213–2217.

Hertog, M. G. L., Hollman, P. C. H., & Katan, M. B. (1992). Content of potentially anticarcinogenic flavonoids of 28 vegetables and 9 fruits commonly consumed in the Netherlands. *Journal of Agricultural and Food Chemistry, 40*, 2379–2383.

Hunter, K. J., & Fletcher, J. M. (2002). The antioxidant activity and composition of fresh, frozen, jarred and canned vegetables. *Innovative Food Science and Emerging Technologies, 3*, 399–406.

Ismail, A., Zamaliah, M. M., & Chin, W. F. (2004). Total antioxidant activity and phenolic content in selected vegetables. *Food Chemistry, 87*, 581–586.

ISO/6557-2. *Fruits, vegetables and derived products - Determination of ascorbic acid content. Part 2. Routine methods*. International Organization for Standardization, Case Postale 56, CH-1211, Geneve 20, Switzerland, 1984.

Jaworska, G., Kmiecik, W., & Maciejaszek, I. (2001). Comparison of the quality of canned spinach (*Spinacia Oleracea* L.) and New Zealand spinach (*Tetragonia Expansa* MURR). *Electronic Journal of Polish Agricultural Universities 4(2),* Available Online: http://www.ejpau.media.pl/volume4/issue2/food/art-04.html.

Jaworska, G., & Kmiecik, W. (2000). Comparison of the nutritive value of frozen spinach and New Zealand spinach. *Polish Journal of Food and Nutrition Sciences, 9/50*(4), 79–84.

Leskova, E., Kubikova, J., Kovacikova, E., Kosicka, M., Porubska, J., & Holcikova, K. (2006). Vitamin losses: Retention during heat treatment and continual changes expressed by mathematical models. *Journal of Food Composition and Analysis, 19*(4), 252–276.

Lin, L. Z., & Harnly, J. M. (2009). Identification of the phenolic components of collard greens, kale, and Chinese broccoli. *Journal of Agricultural and Food Chemistry, 57*, 7401–7408.

Ninfali, P., & Bacchiocca, M. (2003). Polyphenols and antioxidant capacity of vegetables under fresh and frozen conditions. *Journal of Agricultural and Food Chemistry, 51*, 2222–2226.

Oboh, G. (2005). Effect of blanching on the antioxidant properties of some tropical green leafy vegetables. *LWT- Food Science and Technology, 38*, 513–517.

Petersen, M. A. (1993). Influence of sous vide processing, steaming and boiling on vitamin retention and sensory quality in broccoli flowerets. *Zeitschrift fur Lebensmittel-Untersuchung und Forschung, 197*, 375–380.

Podsędek, A. (2007). Natural antioxidants and antioxidant capacity of Brassica vegetables: A review. *LWT – Food Science and Technology, 40*, 1–11.

Ponne, C. T., Baysal, T., & Yuksel, D. (1994). Blanching leafy vegetables with electromagnetic energy. *Journal of Food Science, 59*, 1037–1041. 1059.

Price, K. R., Casuscelli, F., Colquhoun, I. J., & Rhodes, J. C. (1998). Composition and content of flavonol glycosides in broccoli florets (Brassica olearacea) and their fate during cooking. *Journal of the Science of Food and Agriculture, 77*, 468–472.

Puupponen-Pimiä, R., Häkkinen, S. T., Aarni, M., Suortti, T., Lampi, A. M., Eurola, M., et al. (2003). Blanching and long-term freezing affect various bioactive compounds of vegetables in different ways. *Journal of Science and Food Agriculture, 83*, 1389–1402.

Re, R., Pellegrini, N., Proteggente, A., Pannala, A., Yang, M., & Rice-Evans, C. (1999). Antioxidant activity applying an improved ABTS radical cation decolorization assay. *Free Radical Biology and Medicine, 26*(9/10), 1231–1237.

Record, I. R., Dreosti, I. E., & McInerney, J. K. (2001). Changes in plasma antioxidant status following consumption of diets high or low in fruit and vegetables or following dietary supplementation with an antioxidant mixture. *British Journal of Nutrition, 85*, 459–464.

Roy, M. K., Juneja, L. R., Isobe, S., & Tsushida, T. (2009). Steam processed broccoli (Brassica oleracea) has higher antioxidant activity in chemical and cellular assay systems. *Food Chemistry, 114*, 263–269.

Selman, J. D. (1994). Vitamin retention during blanching of vegetables. *Food Chemistry, 49*, 137–147.

Sikora, E., Cieślik, E., Leszczyńska, T., Filipiak-Florkiewicz, A., & Pisulewski, P. (2008). The antioxidant activity of selected cruciferous vegetables subjected to aquathermal processing. *Food Chemistry, 107*, 55–59.

Turkmen, N., Sari, F., & Velioglu, S. (2005). The effect of cooking methods on total phenolics and antioxidant activity of selected green vegetables. *Food Chemistry, 93*, 713–718.

Wachtel-Galor, S., Wong, K. W., & Benzie, I. F. F. (2008). The effect of cooking on *Brassica* vegetables. *Food Chemistry, 110*, 706–710.

Yamaguchi, T., Mizobuchi, T., Kajikawa, R., Kawashima, H., Miyabe, F., & Terao, J. (2001). Radical-scavenging activity of vegetables and the effect of cooking on their activity. *Food Science and Technology Research, 7*(3), 250–257.

Zhang, D., & Hamauzu, Y. (2004). Phenolics, ascorbic acid, carotenoids and antioxidant activity of broccoli and their changes during conventional and microwave cooking. *Food Chemistry, 88*(4), 503–509.