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The effect of heating and fermenting on antioxidant properties of white cabbage

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Abstract

It is widely believed that natural antioxidants found in food are significantly lost during processing. Nevertheless, it was recently dem-onstrated that processed fruits and vegetables may retain their antioxidant activity. In the present work, the changes in the overall anti-oxidant properties as a consequence of fermentation of cabbage and/or heat treatment of cabbage juices and extracts were studied. Fermentation processes as well as heat treatment increased the initial values of antioxidant activity. While a decrease in the antioxidant potential of sauerkraut juice was found for short heat treatments, a partial recovery of these properties was observed by prolonging heat-ing periods. The TLC analysis showed that during fermentation and thermal processes, some substances with reactivity towards Folin–Ciocalteu reagent, hence with possible antioxidant activity, were released. We demonstrated that in contrast to common expectation, typical culinary processing of cabbage increases its antioxidant potency. The gain in antioxidant activity of heated samples coincided with the formation of both F–C reagent reactive compounds as well as brown early Maillard reaction products. This information may encourage the consumption of heat processed cabbage, especially that the release of antioxidants during heating may prevent oxi-dation of other food components, e.g. fats.

Keywords: White cabbage; Antioxidant properties; Heat treatment; Fermentation

1. Introduction

Increased consumption of fruit and vegetables has been associated with protection against various diseases, including cancer and cardiovascular diseases. These health promoting properties are associated with non-nutritive phytochemicals displaying a variety of activities either diminishing exposition of human organism on detrimental environmental factors (*e.g.* antioxidants) or boosting endogenous detoxifying mechanism (*e.g.* isothiocyanates). Certain bioactive compounds found in food plants have been known for a long time for their beneficial effects, whereas others have only recently been recognized. Prevention is clearly the most effective strategy to control chronic diseases, so a constant supply of phytochemicals with desirable health benefits beyond basic nutrition is essential to furnish the defensive mechanisms. Since different plants contain different phytochemicals, with specific protective activities, to obtain optimal health benefits it is suggested that humans consume a balanced diet with a variety of phytochemical sources from whole foods, such as fruits, vegetables, and grains as part of whole meals (Liu, 2002).

Food composition tables, which are necessary tools for epidemiological and nutritional studies, are mainly representative of foodstuffs consumed in their raw state. In reality, only a small amounts of fruits and vegetables are

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consumed in their raw state, whilst most of them need to be processed for safety, quality and economic reason. The evaluation of the influence of food processing is a key factor while establishing technological conditions that enable to preserve or improve original activity and bioavailability of naturally occurring antioxidants. Understanding the consequences of food processing on food composition, is one of the important steps to a correct interpretation of study results regarding dietary habits, nutrition and human health (Nicoli, Anese, & Parpinel, 1999).

There is an ample literature concerning the level of phytochemicals in raw fruits and vegetables, but only some reports appeared that describe the changes of composition of bioactive compounds during processing (Ciska & Pathak, 2004; Kidmose, Yang, Thilsted, Christensen, & Brandt, 2006; Oerlemans, Barret, Suades, Verkerk, & Dekker, 2006). Preservation methods are generally believed to be responsible for a depletion of naturally occurring antioxidants in food. The most recent report by Oszmianski, Wolniak, Wojdylo, and Wawer (2007) revealed that also the way of production may have important implications as regards antioxidative properties. This group demonstrated that cloudy apple juice is better source of natural antioxidants than clear apple juice. Consequently, processed fruits and vegetables would be expected to have lower health protecting capacity than fresh ones. This is because, up to now, only selected and rather unstable antioxidants of nutritional interest (*e.g.* ascorbic acid) have been commonly assessed as indicators of processing damage. However, in some cases, processing causes loss or no change to the content and activity of naturally occurring antioxidants (Amin & Lee, 2005; Davey et al., 2000; Hong, Barrett, & Mitchell, 2004; Oszmianski et al., 2007; Puupponen-Pimia et al., 2003) but sometimes, it leads to the formation of novel compounds with antioxidant activity Dewanto, Adom, & Lui, 2002; Dewanto, Wu, & Liu, 2002; Durmaz & Alpaslan, 2007; Nicoli, Anese, Parpinel, Franceschi, & Lerici 1997; Turkmen, Sari, & Velioglu, 2005; Turkmen, Sari, Poyrazoglu, & Velioglu, 2006).

Cruciferous vegetables, such as cabbage, are among the most important dietary vegetables consumed in Poland and other Central European countries owing to their availability at local markets, low cost and consumer preference. It is estimated that in Poland the annual cultivation of cabbage constitutes about 30% of the total production of ground vegetables (Polish Central Statistical Office, 2006). White cabbage is consumed both raw and processed in different ways, *e.g.* stewed or fermented. Fermented cabbage known as sauerkraut is very popular also in Germany and Austria as its name, originating from German language, implies. However, it is also frequently consumed in the United States, Canada, and Russia, especially throughout the winter period, as a properly performed process guarantees good quality of the product during storage. Despite being an important dietary component, there are very limited data on the influence of fermentation process on antioxidant properties of cabbage. Similarly, on the influence of heat treatment on antioxidant activity of cabbage relatively little has been reported. Most of the work so far has been carried out on blanched cabbage (Amin and Lee, 2005; Puupponen-Pimia et al., 2003). Surprisingly, although prolonged cooking is a typical way of preparing meals containing cabbage, there is no data on antioxidant activity of cabbage heated for longer periods than 15 min.

The main objective of this study was to determine changes in antioxidant activity of cabbage during long-term heat treatment and two weeks of fermentation process, the conditions matching typical culinary processing of this vegetable.

2. Materials and methods

2.1. Chemicals

2,2′-Azinobis(3-ethylbenzothiazoline-6-sulfonic acid) (ABTS); 1,1-diphenyl-2-picrylhydrazyl (DPPH); 6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid (Trolox) and Folin–Ciocalteu reagent were from Sigma (Germany). All reagents were of analytical grade.

2.2. Samples and their preparation

Fresh white cabbage *Brassica, Oleracea, Capitata, Cruciferae* (20 kg) was purchased in a wholesale shop supplying the area of Gdansk (Northern Poland) in vegetables. After removal of outer leaves, cabbage heads were cut in a shredder into ~2 mm thick strips. The part of shredded cabbage (5 kg) was blended, and the juices were squeezed out from the pulps. Another portion of cabbage (5 kg) was freeze-dried and ground to powder. The rest of shredded cabbage was submitted to fermentation process. The shredded cabbage (9 kg) was mixed with 20 g kg^{-1} of NaCl and fermented in a traditional stoneware pots for 2 weeks. During this process, the portions of cabbage (~2 kg) were collected after 4, 7, 11 and 14 days. Each portion of sauerkraut was divided on semi, one part was freeze-dried and another one was blended, and then squeezed out to collect juice.

For heat treatment, the fresh cabbage juice and sauerkraut juice (14 days fermentation) were aliquoted as 4 mL portions into glass vials, sealed to avoid evaporation and placed in a thermoreactor TR 300 (Merck) set on 100 °C. The heating was carried out up to 15 h. After each hour, the samples (4 mL) of the fresh cabbage juice and sauerkraut juice were collected in triplicate and cooled to room temperature. For ABTS and DPPH assays the juices were directly used, while for TLC analysis, 1 mL of each sample collected was freeze-dried and extracted with methanol (1 mL).

For heat treatment, the powders of lyophilized fresh cabbage or sauerkraut (14 days fermentation) were distributed as 1 g portions to glass vials, sealed to prevent loss of volatile compounds and placed in a thermoreactor TR 300 (Merck) set on 100 °C. The heating was carried up to

80 min. Every 10 min, the samples of the roasted cabbage and sauerkraut (1 g) were collected in triplicate. After cooling to room temperature, the samples were extracted twice with 4 mL of methanol. The extracts were then submitted to ABTS, DPPH and F–C assays and analyzed by TLC method.

2.3. Determination of free radical scavenging activity

The free radical scavenging activity of cabbage juices, cabbage extracts and the standards (ascorbic acid or Trolox) was determined according to two complementary ABTS and DPPH free radical scavenging assays described by Huang, Ou, and Prior (2005). For the first assay, $ABTS^{\cdot+}$ radical cation was generated by the interaction of ABTS (7 mmol L^{-1}) and $K_2S_2O_8$ (2.45 mmol L^{-1}). The mixture was allowed to stand at room temperature for 12 h to give a dark green solution. This solution was diluted (with PBS buffer for analysis of juices or with methanol for analysis of extracts) until the absorbance reached 0.7 at 734 nm. For measurement, 1 mL of this $ABTS^{\cdot+}$ solution was mixed with 10 μL of cabbage juices or methanolic extracts. The absorbance was read 6 min after mixing at 734 nm. Trolox solutions (0–2 μmol mL^{-1}) were used to generate the standard line. In the case of second assay, 2.9 mL of DPPH solution in methanol (40 mg L^{-1}) was mixed with 0.1 mL of juices or methanolic extracts. The progress of reaction was monitored at 515 nm until the absorbance was stable.

Radical scavenging capacity was expressed as Trolox equivalents per mL of juice or per gram of dry matter and as the inhibition percentage for both methods (Singh, Chidamdara, & Jayaprakasha, 2002). The antiradical activity of standard (ascorbic acid) was expressed as EC_{50} parameter, defined as the concentration that causes a decrease in the initial ABTS or DPPH concentration by 50%.

Additionally, antioxidative potential was measured spectrophotometrically with the aid of Folin–Ciocialteu's phenol reagent (Merck, Germany). Samples of juices or methanolic extracts (0.1 mL) were mixed with commercial phenol reagent diluted 1:9 with water (1 mL). After 10 min, the absorption of samples was measured at 750 nm. Trolox was used to generate the standard line.

2.4. TLC analyses

Methanolic extracts from cabbage were applied using glass capillary onto plates coated with silica gel 60 F_{254}, 0.25 mm (Merck, Germany). Mobile phase was a mixture of chloroform and methanol (9:1). Detection of separated compounds was achieved by spraying plates with Folin–Ciocalteu phenol reagent (F–C reagent) diluted with water 1:1 [v/v], as described earlier (Kusznierewicz, Wolska, Bartoszek, & Namiesnik, 2005).

2.5. Browning pigment formation (BPF)

For this assay, extracts were diluted with methanol (1:9) to reach the measurable level of absorption. BPF was determined by measuring the absorbance of diluted fresh cabbage and sauerkraut methanolic extracts at 420 nm for appropriate heating time points.

3. Results and discussion

3.1. Effect of fermentation period on the antioxidant activity of cabbage

The influence of time of fermentation process on total antioxidant properties of cabbage juice and cabbage methanolic extracts is shown in Fig. 1. The results of ABTS and DPPH assays indicate that during spontaneous fermentation the antioxidant activity of cabbage gradually increases and reaches plateau after about 10 days. Reyes, Villarreal, and Cisneros-Zevallos (2007) reported that antioxidant activity of cabbage increases during wounding. In our experiments, cabbage was shredded before fermentation, so wounding could be one of the reasons of initial rise of antioxidant activity. The elevated antioxidant capacity of

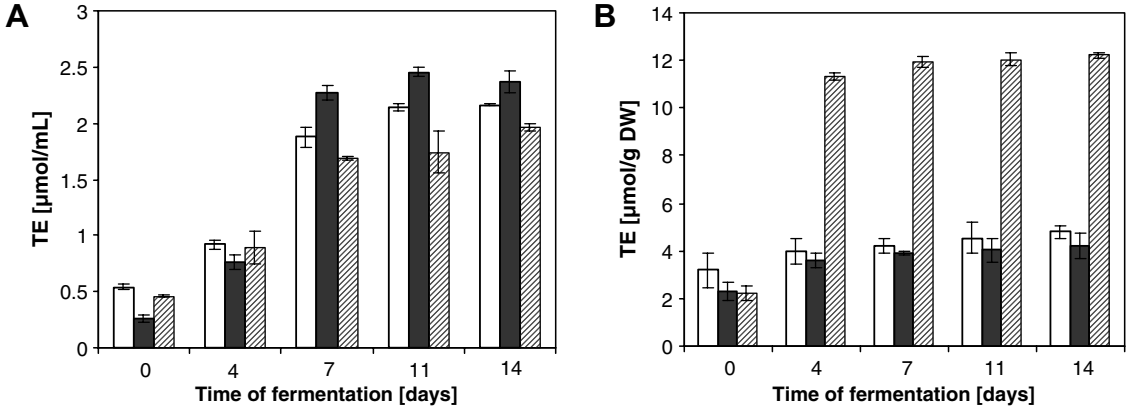

Fig. 1. The level of total antioxidant activity in (A) – cabbage juice (TE μmol mL^{-1}) and (B) – methanolic extracts of cabbage (TE μmol g^{-1} DW) as determined by ABTS □; DPPH ■ and F–C ▨ assays. Results represent means ± SD of three independent experiments.

sauerkraut probably combines effects of wounding and chemical processes incurred by lactic bacteria. In all our experiments that have been carried out over past few years, such an increase was evident for spontaneously fermented sauerkraut (Bartoszek, Forc, & Grzeskowiak, 2002; Kusznierewicz et al., 2005). Although fermenting conditions sometimes lead to enhancement of antioxidant properties (as well as vitamin C contents) of cabbage, it is not necessarily the case. The earlier data from Finnish group (Tolonen et al., 2004) and very recent report by Skapska et al., 2007) show that strictly controlled fermentation by some isolated strains of lactic bacteria does not change antioxidant potency of final sauerkraut compared to fresh vegetable. Hence, the conditions of sauerkraut production strongly influence its eventual health promoting properties.

The gradual growth of antioxidant potential in the case of juices is much more evident than of methanolic extracts. The fresh cabbage (0 days of fermentation), both juice and methanolic extract, shows the lowest antioxidant potential, respectively 0.54 ± 0.02 TE μmol mL^{-1} and 3.17 ± 0.71 TE μmol g^{-1} DW in ABTS assay, and 0.26 ± 0.03 TE μmol mL^{-1} and 2.31 ± 0.39 TE μmol g^{-1} DW in DPPH assay. The sauerkraut juice collected after 14 days of fermentation exhibits about three times higher antioxidant potential in ABTS assay (1.74 ± 0.15 TE μmol mL^{-1}) and 7 times higher in DPPH assay (1.88 ± 0.05 TE μmol mL^{-1}) relatively to initial value determined for fresh cabbage juice (Fig. 1A). The highest antioxidant activity among methanolic extracts is exhibited by the samples collected after 2 weeks of fermentation, that is in ABTS assay: 4.79 ± 0.26 TE μmol g^{-1} DW and in DPPH assay: 4.20 ± 0.23 TE μmol g^{-1} DW (Fig. 1B). The measured antioxidant properties obtained in ABTS assay are repeatedly higher than these obtained in DPPH assay. The increase of vitamin C content upon cabbage fermentation is known and could account for the higher values of antioxidant activity of cabbage juice in DPPH test, because the parameter EC_{50} of ascorbic acid is lower in DPPH test (0.12 mg mL^{-1}) than in ABTS test (0.22 mg mL^{-1}). Similar trends are observed for antioxidant activity in F–C assay. Interestingly, methanol seemed to be very effective in extracting F–C reactive compounds (Fig. 1, panel B). A very good correlation was observed between the levels of Trolox equivalents determined by ABTS and DPPH scavenging assays and F–C antioxidant assay. The calculated correlation coefficients for antioxidant analyses were very high. In the case of juices, the R^2 coefficients amounted to 0.998, 0.967 and 0.981 for DPPH/ABTS, DPPH/F–C and ABTS/F–C, respectively. For methanolic extracts, corresponding values were: 0.943, 0.954 and 0.811.

We were also interested whether the increased antioxidant activity of sauerkraut resulted solely from the changes in vitamin C content (fresh – 0.3 mg mL^{-1}; sauerkraut – 1.2 mg mL^{-1}) or was also enhanced due to formation of other compounds capable of neutralizing free radicals. Therefore, the cabbage samples were analyzed by TLC as described by Kanner, Frankel, Granit, German, and Kinsella (1994) for natural food samples. The patterns of Folin–Ciocalteu reactive spots in the TLC chromatogram of juice samples and methanolic extracts of cabbage fermented for different periods of time are shown in Fig. 2. The Folin–Ciocalteu reagent (F–C) has for many years been believed to be a measure of total phenolics in natural products. However nowadays, it has been recognized that it is involved in the basic mechanism of oxidation/reduction reaction and, as such can react with, hence reveal the presence of any substance with antioxidant properties (Prior, Wu, & Schaich, 2005). So the use of F–C reagent for the TLC chromatogram visualization enabled us to follow the general profile of antioxidants present in analyzed samples rather than to selectively detect phenolics. The obtained results presented in Fig. 2 suggest that during cabbage fermentation some compounds other than vitamin C, the latter cannot be resolved under chromatographic conditions applied, with antioxidant properties are released. Since no such spots (with corresponding R_f values) are observed in chromatograms of fresh unfermented cabbage, it follows that they must arise during fermentation process.

3.2. Effect of heat treatment on the antioxidant activity of fresh cabbage and sauerkraut

Polish traditional cuisine as well as other national cuisines in Central Europe (German, Chech and Austrian) abounds in dishes made from cabbage or with addition of cabbage. Some of the most popular dishes are made from either fresh cabbage or sauerkraut or their mixture stewed with meat even for period of several hours. We investigated whether this type of processing abolishes the ability of this vegetable to scavenge free radicals. It is very important

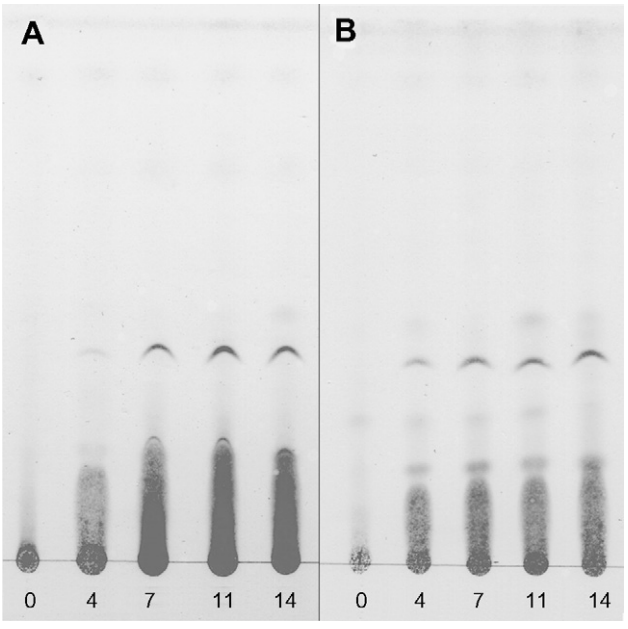

Fig. 2. TLC chromatograms visualized by F–C reagent of (A) cabbage juice and (B) methanolic cabbage extracts. The numbers at the bottom (0, 4, 7, 11 and 14) refer to the days of fermentation.

from dietary point of view, as both muscle proteins as well as fats present in meat are easily oxidized upon heating in oxygen atmosphere which leads to the formation of toxic substances and diminishes nutritional value of meat.

The overall antioxidant properties of fresh cabbage and sauerkraut juices heated at 100 °C for up to 15 h were quantitatively assessed by measuring their free radical scavenging capacity by ABTS and DPPH assays and antioxidant potency by F–C assay. Table 1 shows the determinations obtained for heated cabbage juices expressed as μmol Trolox equivalents (TE) per mL of juice. The antiradical determination, but as percent of inhibition, is shown graphically in Fig. 3A. The scavenging effect of fresh cabbage juice on ABTS and DPPH radicals changed significantly with the heating time (Table 1). While unheated samples showed the least activity, 1 h heated samples displayed scavenging capacity almost doubled and maintained growing tendency almost over whole period studied (15 h). Similar results were reported for tomato juice where samples heated for 15 h and longer, showed chain breaking activity values progressively higher than those measured for the unheated tomato samples (Anese, Manzocco, Nicoli, & Lerici, 1999). In the case of sauerkraut juice, this kinetics exhibited two phases. In the first phase, a considerable reduction in antioxidant activity during first 4 h of heating was observed (Fig. 3A). It was not unexpected as natural antioxidants present in the sauerkraut aqueous phase, mainly ascorbic acid, are known to readily undergo thermal degradation and/or consumption in the Maillard reaction pathway (Davies & Wedzicha, 1994). Anese et al. (1999) reported reduction of the ascorbic acid concentration in the tomato samples close to 90% after 4 h heating. The second phase begun after 4 h of heating, when the antioxidant properties of sauerkraut juice started to rise and kept to increase for about 10 h of heating. Nonetheless, after 15 h of heating, the scavenging effect of sauerkraut juice was lower than that of fresh cabbage juice heated for the same period of time and still lower than unheated sauerkraut juice.

Table 2 contains values of antioxidant activity of methanolic extracts obtained from freeze-dried fresh cabbage and sauerkraut that was roasted (*i.e.* heated as a powder) for different periods of time, expressed as μmol Trolox equivalents (TE) per g of dry matter. The same data recalculated to percent of inhibition are shown graphically in Fig. 3B. Roasting of dry lyophilizates was performed at 100 °C for 80 min, then the samples were extracted with methanol as described under Section 2. The scavenging effect of fresh cabbage extracts as well as sauerkraut extracts on ABTS and DPPH radicals increased significantly with the roasting time. The same trend was observed for antioxidant activity in F–C assay (Table 2). Thus, also in this case, the exposition to high temperature promoted release and/or formation of free radical scavengers and antioxidants. Fresh cabbage samples heated for 30 min showed antiradical activity 6 and 4 times higher in ABTS and DPPH assays, respectively, than those measured for the unheated samples. The antiradical properties of sauerkraut after 30 min increased four times in ABTS test and two times in DPPH test. Methanol turned out to be particularly effective in extracting antioxidants (as in the case of fermentation) and antioxidant activity measured by F–C assay compared to starting material increased almost 15 times for fresh cabbage and twice for sauerkraut samples.

The increase in radical scavenging power of cabbage samples exposed to high temperature can be explained by the formation of some reductant compounds formed throughout heating, as compounds with such chemical

Table 1
Total antioxidant activity of thermally treated fresh cabbage and sauerkraut juices determined by ABTS, DPPH and F–C assays

Time of heating (h)	Antioxidant activity of juices (TE μmol/mL)					
	Fresh cabbage			Sauerkraut		
	ABTS	DPPH	F–C	ABTS	DPPH	F–C
0	0.42 ± 0.14	0.28 ± 0.05	0.71 ± 0.03	1.73 ± 0.14	1.88 ± 0.05	1.97 ± 0.03
1	0.84 ± 0.03	0.49 ± 0.03	–	1.44 ± 0.07	1.21 ± 0.04	–
2	1.07 ± 0.09	0.60 ± 0.04	1.32 ± 0.09	1.31 ± 0.17	1.04 ± 0.04	1.17 ± 0.01
3	1.38 ± 0.09	0.69 ± 0.02	–	1.11 ± 0.18	0.81 ± 0.09	–
4	1.60 ± 0.10	0.88 ± 0.06	1.60 ± 0.11	0.79 ± 0.15	0.71 ± 0.04	0.87 ± 0.05
5	1.90 ± 0.06	1.06 ± 0.08	–	1.01 ± 0.06	0.81 ± 0.04	–
6	2.23 ± 0.29	1.17 ± 0.09	2.12 ± 0.11	1.08 ± 0.17	0.86 ± 0.02	1.04 ± 0.01
7	2.34 ± 0.34	1.27 ± 0.06	–	1.15 ± 0.03	0.89 ± 0.04	–
8	2.79 ± 0.12	1.48 ± 0.08	2.65 ± 0.11	1.24 ± 0.06	0.95 ± 0.01	1.15 ± 0.07
9	2.93 ± 0.19	1.59 ± 0.10	–	1.34 ± 0.17	0.98 ± 0.01	–
10	2.93 ± 0.18	1.72 ± 0.06	2.97 ± 0.21	1.37±0.15	1.06 ± 0.04	1.15 ± 0.11
11	3.28 ± 0.08	1.88 ± 0.06	–	1.52 ± 0.07	1.13 ± 0.06	–
12	3.31 ± 0.04	1.90 ± 0.07	3.04 ± 0.19	1.46 ± 0.03	1.04 ± 0.02	1.14 ± 0.02
13	3.39 ± 0.44	1.95 ± 0.04	3.10 ± 0.37	1.49 ± 0.09	1.01 ± 0.06	1.21 ± 0.11
14	3.45 ± 0.16	1.95 ± 0.06	2.95 ± 0.29	1.49 ± 0.20	0.98 ± 0.02	1.21 ± 0.13
15	3.51 ± 0.17	2.01 ± 0.07	3.17 ± 0.09	1.54 ± 0.12	1.06 ± 0.03	1.24 ± 0.03

Values are expressed as means ± standard deviation of three independent experiments. Total antioxidant activity of all samples is expressed as Trolox equivalents (TE).

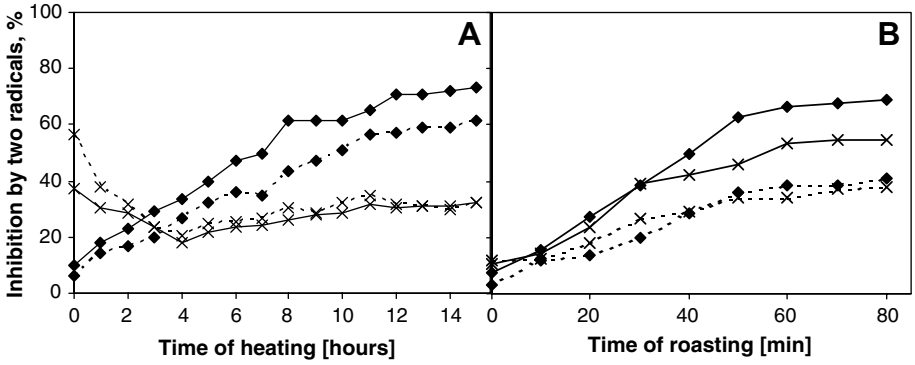

Fig. 3. The influence of time of heating on antioxidant properties of fresh cabbage and sauerkraut juices (A) and powdered freeze-dried fresh cabbage and sauerkraut (B) as determined by ABTS and DPPH assays (Inhibition, %). Results represent means of three independent experiments. The SD values were in the range from 0.4% to 6.8%. (–♦–, fresh cabbage: ABTS; --♦--, fresh cabbage: DPPH; –×– ,sauerkraut: ABTS; --×--, sauerkraut: DPPH.)

Table 2
Total antioxidant activity of thermally treated powder of freeze-dried fresh cabbage and sauerkraut determined by ABTS, DPPH and F–C assays

Time of roasting (min)	Antioxidant activity of lyophilized cabbage (TE μmol/g DW)					
	Fresh cabbage			Sauerkraut		
	ABTS	DPPH	F–C	ABTS	DPPH	F–C
0	3.18 ± 0.71	1.73 ± 0.20	2.14 ± 0.29	4.79 ± 0.26	4.20 ± 0.53	9.20 ± 1.29
10	7.14 ± 1.01	4.27 ± 0.22	10.35 ± 1.56	6.58 ± 0.68	4.41 ± 0.14	11.48 ± 1.05
20	13.11 ± 0.80	4.89 ± 0.41	21.73 ± 0.67	11.37 ± 1.54	5.93 ± 0.32	17.72 ± 0.42
30	18.37 ± 2.51	7.41 ± 1.43	30.61 ± 0.64	18.55 ± 0.97	9.19 ± 0.35	21.82 ± 0.04
40	24.02 ± 1.34	9.91 ± 0.93	35.01 ± 2.26	20.28 ± 3.44	10.02 ± 1.38	22.06 ± 1.19
50	30.40 ± 2.63	13.26 ± 0.55	37.36 ± 1.24	22.10 ± 2.36	12.25 ± 1.11	22.79 ± 0.09
60	31.38 ± 1.27	14.16 ± 0.21	44.57 ± 5.83	24.79 ± 2.38	13.15 ± 0.46	25.29 ± 0.46
70	31.92 ± 1.57	14.55 ± 1.15	45.82 ± 3.14	25.07 ± 2.54	14.21 ± 0.73	28.28 ± 0.97
80	32.57 ± 1.03	14.93 ± 1.53	49.33 ± 5.83	25.36 ± 2.71	14.26 ± 0.72	27.97 ± 0.22

Values are expressed as means ± standard deviation of three independent experiments. Total antioxidant activity of all samples is expressed as Trolox equivalents (TE).

property were reported to be intermediates of Maillard reactions (MRPs) (Hodge, 1953). Positive correlation between the intensity of the colour and antioxidant properties has been found in the case of the development of Maillard reactions in model systems and in studied food products (Anese et al., 1999; Manzocco, Calligaris, Mastrocola, Nicoli, & Lerici, 2001; Turkmen et al., 2006).

Fig. 4 shows the changes in the antioxidant activity and brown pigment formation (BPF) in methanolic extracts obtained from freeze-dried fresh cabbage and sauerkraut roasted at 100 °C for various time periods. High correlation coefficients calculated after plotting the data for antioxidant activity versus BPF at 100 °C indicated that there is a strong relationship between both criteria (Table 3).

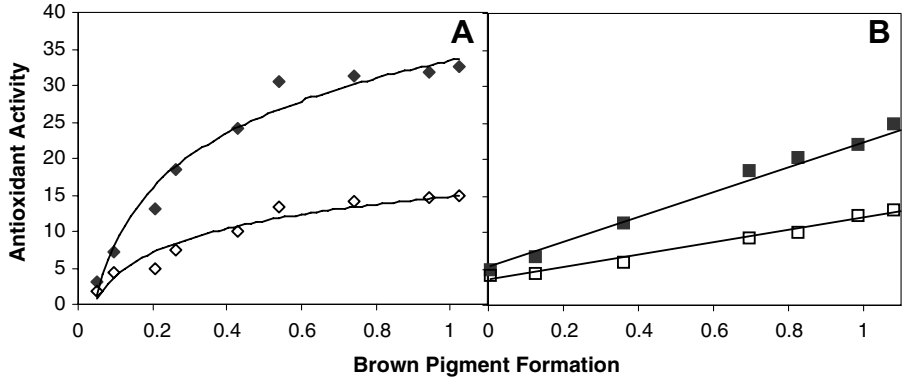

Fig. 4. The relationship between antioxidant activity (determined by ABTS and DPPH assays, TE μmol g^{-1} DW) and brown pigment formation (absorption, $\lambda = 420$ nm) of methanolic extracts obtained from roasted fresh cabbage (A) and sauerkraut (B) lyophilizates. (♦, fresh cabbage: ABTS; ◇, fresh cabbage: DPPH; ■, sauerkraut: ABTS; □, sauerkraut: DPPH).

Table 3
Regression equations and correlation coefficients (R^2) of antioxidant activity determined by ABTS and DPPH assays as a function of BPF values for extracted lyophilizates of roasted at 100 °C fresh cabbage and sauerkraut

Sample/assay	Regression equations	R^2
Fresh cabbage/ABTS	$y = 10.852\ln(x) + 33.393$	0.970
Fresh cabbage/DPPH	$y = 4.758\ln(x) + 14.739$	0.946
Sauerkraut/ABTS	$y = 16.985x + 5.237$	0.982
Sauerkraut/DPPH	$y = 8.589x + 3.459$	0.988

As seen in Fig. 4, the increase in antioxidant activity was accompanied by the increase in browning of samples due to MRPs. While antioxidant activity increased linearly with increasing heating time and BPF for sauerkraut, logarithmic increase in the case of fresh cabbage was observed (Fig. 4A). This can be attributed to the complexity of non-enzymatic browning reactions because they involve different compounds and proceed through different chemical pathways depending on composition of product and processing conditions (Durmaz & Alpaslan, 2007). Though, no data have been found on the antioxidant activity and colour changes during heating of cabbage, the study by Anese et al. (1999) carried out for tomato juice, demonstrated that heating at 95 °C for up to 30 h caused a progressive increase in overall antioxidant potential of the tomato juice which was accompanied by increasing optical density. Durmaz and Alpaslan (2007) found that in roasted dried kernel flour, contrary to browning degree, radical scavenging power, reducing power and total phenolic content did not increase linearly but showed a maximum for 10 min of roasting. Turkmen et al. (2006) reported that for honey the antioxidant activity and BPF increased with treatment temperature and time of heating. Similarly to our findings, these authors showed that antioxidant activity was correlated with increased browning of the honey samples.

The TLC chromatograms of heated cabbage juice samples are shown in Fig. 5. The TLC chromatograms were visualized by spraying the plates with F–C reagent. The

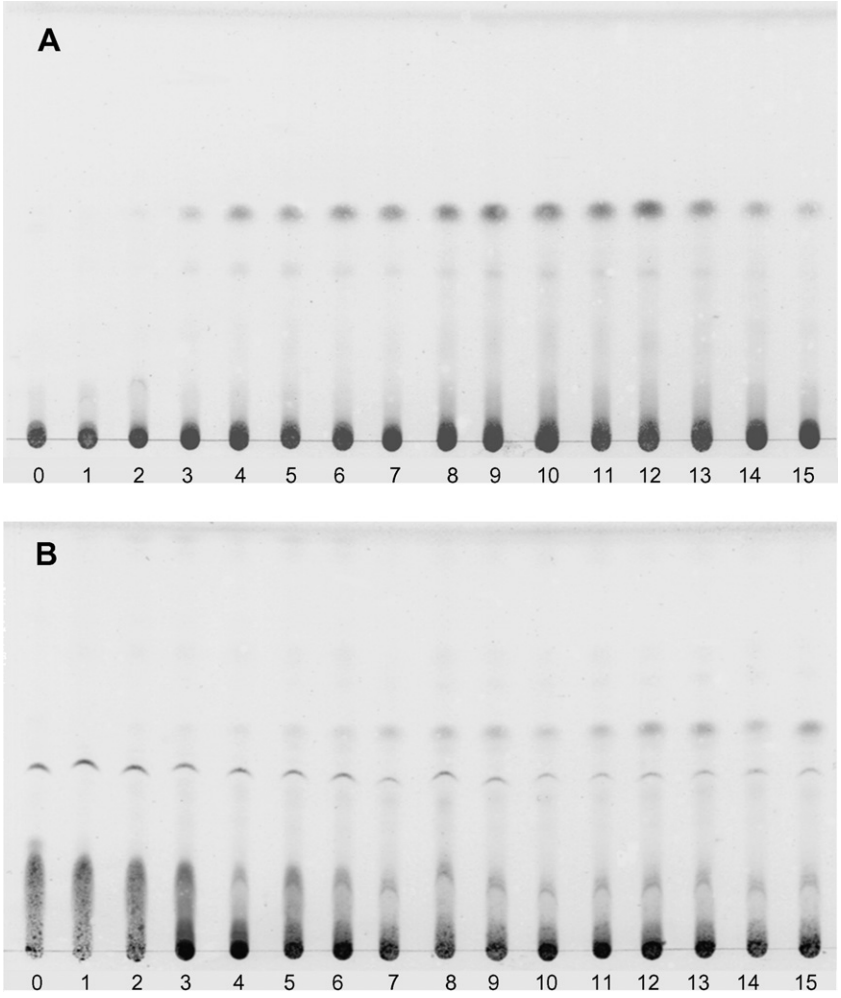

Fig. 5. TLC chromatograms of thermally treated fresh cabbage juice (A) and sauerkraut juice (B) visualized by F–C reagent. The numbers at the bottom (0–15) refer to the hours of heating.

chromatographic patterns observed confirmed considerable differences in quantitative and qualitative composition between heated and unheated cabbage samples. The samples of heated juices and extracts from roasted cabbages (latter not shown) contained considerably greater amounts of compounds characterised by higher R_f values which in the case of chromatographic conditions applied indicates more non-polar substances. For fresh cabbage samples (Fig. 5A, line 0), almost no compounds reacting with F–C reagent were observed, while heated fresh cabbage samples gave rise to spots whose colour became more intensive upon heating (Fig. 5A, lines 1–15). Unheated sauerkraut samples were characterised by the presence of compounds emerging during fermentation process (Fig. 5B; line 0). During heating, in sauerkraut samples some new compounds, present also in heated fresh cabbage samples appeared (Fig. 5B lines 1–15). Similar pattern of F–C reactive chromatographic spots was observed in extracts from roasted cabbage. These results suggest that in the case of cabbage, thermal treating can be the reason of formation of novel compounds with antioxidant activity. Puupponen-Pimia et al. (2003) reported in blanched cabbage the increase of antioxidant activity and higher concentration of some bioactive components, such as flavonol and sterols.

4. Conclusion

Chemopreventive potential exhibited by plant-borne foods has become one of the major fields of health related research recently, as it might help to diminish the global burden of diseases including cancer by implementing specific dietary recommendations. A number of substances displaying anticarcinogenic properties have been characterized: genistein found in soy, lycopene in tomatoes, epigallocatechins in tea, sulforaphane in broccoli, resveratrol in grapes, to name only a few most extensively investigated.

Chemopreventive potential of cabbage, the most frequently consumed vegetable around the world, has drawn surprisingly little attention so far. White cabbage, fresh, cooked or fermented with lactic bacteria is an ample ingredient of Central European diet. Therefore, if it contains health-promoting components, their impact may also be considerable. There are many reasons to presume that this edible plant may benefit human health in a number of ways. Here, we concentrated on antioxidative properties of cabbage which are important not only because they prevent or at least diminish exposition of human organism on ROS that would otherwise attack vital biomolecules, but in addition dietary antioxidants are able to protect other food components from oxidative deterioration. The present study showed that fermentation process as well as heat treatment, both short and prolonged, greatly improve the antioxidant properties of cabbage. The heat processing seemed to compensate the loss of natural antioxidants by the formation of non-nutrient antioxidants such as MRPs. Our results revealing that cabbage increases its antioxidative potential upon heating seems particularly important for the inhibition of primary oxidation product formation in fat food system. Particularly vulnerable are devoid of endogenous antioxidants animal fats, a typical component of cabbage containing dishes. Their thermooxidative degradation leads to the formation of numerous toxic, also genotoxic, compounds such as peroxides and aldehydes. The traditional cooking of animal fats with cabbage may thus slow down these processes. Indeed, it was observed that the presence of cabbage (both fresh cabbage and sauerkraut) components protected lard against oxidation for several hours of heating at 100 °C (Szukalska, Tynek, Debecka, & Papiernik, 2005). In accord with our experiments, fresh cabbage provided more effective antioxidative barrier.

Although, the fermentation of cabbage by lactic bacteria increases antioxidative activity of this vegetable, upon cooking the antioxidative potential of sauerkraut decreases, while from fresh cabbage substances with such activity seem to be gradually released. This gradual release of antioxidants from fresh cabbage turns out to be more beneficial as it protects more efficiently other food components liable to oxidative degradation, and as we have show previously also the cells exposed to oxidative stress (Kusznierewicz et al., 2007) than sauerkraut displaying higher initial antioxidative potential.

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