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Research article

Effect of thermal processing on antioxidant power and thiosulfinate content in Brussels sprouts juices

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Abstract: *The aim of this study was to elucidate the influence of thermal processing at 22-95°C on the antioxidant ability of Brussels sprouts juice and its thiosulfinate content. The antioxidant power was determined by FRAP, Folin-Ciocalteu and DPPH radical scavenging methods. Thiosulfinate concentration was assayed by Han's procedure. It was shown that the increase of processing temperature decreased of the antioxidant ability determined by Folin-Ciocalteu method. However, FRAP and DPPH scavenging showed increase by 20% of the antioxidant power in the final heating step (95°C). The increasing processing temperature resulted in decreasing thiosulfinate concentration until the total loss. This proved that the thiosulfinate present in Brussels sprouts juice is thermolabile compound (not resistant for high temperature). The total loss of the thiosulfinate in high temperature did not reflect the change in antioxidant power. This fact indicated that there was no impact of the thiosulfinate on antioxidant abilities of Brussels sprouts juice or the impact was insignificant among the abundance of other antioxidants. The results showed a good correlation between FRAP and DPPH assays of antioxidant power determination in contrast to correlation with the data of Folin-Ciocalteu procedure.*

Keywords*: Brussels sprouts, antioxidant power, thiosulfinate, thermal processing, DPPH.*

Introduction

Plants of *Brassica* genus include vegetable members commonly used for consumption. Representatives of them are cabbage, cauliflower, broccoli, Brussels sprouts, kale. These vegetables are known for their antioxidant and anticarcinogenic properties [1-3]. Brassicas contain specific groups of bioactive compounds such as glucosinolates and methyl-L-cysteine sulfoxide. The sulfoxides are frequent components present in many plants (*e.g.* onion, garlic, asparagus) [4, 5] but the brassicas contain only one of them methyl-L-cysteine sulfoxide (metiin). Metiin is a precursor of methylmethanethiosulfinate (TS) formed in the presence of enzyme cysteine lyase released after tissue destruction:

CH_3-S(O)-CH_2-CH(NH_2)-COOH + H_2O → CH_3-SOH+CH_3-C(O)-COOH + NH_3

2CH_3-SOH → CH_3-S(O)-S-CH_3 + H_2O

At the initial period of the reaction pyruvate, ammonia and methyl sulfenic acid CH_3-SOH are formed. Afterwards CH_3-SOH undergoes rapid transformation into methylmethanethiosulfinate CH_3-S(O)-S-CH_3 [6, 7], which is responsible for the characteristic flavour as well as antibacterial, antifungal, antioxidant brassicas properties. Methylthanethiosulfinate undergos further transformation into dimethylthiosulfonate, dimethylsulfide and polymethylsulfides [7, 8].
The thiosulfinate concentration in Brussels sprouts juice is comparable to other brassicas whereas Brussels sprouts' antioxidant properties are exceptionally high [1, 9].

The health-promoting ability of plant foods strictly depends on their processing history. The conditions of storage, processing and preparation have a significant effect on the content of antioxidant and other pro-health substances content.

The aim of this work was to study the influence of storage and thermal processing on antioxidant ability and thiosulfinate content of Brussels sprouts. The antioxidant properties were determined by the use of Folin-Ciocalteu, FRAP and DPPH radical scavenging methods. The relationships between applied methods were evaluated as well as the impact of thiosulfinate on antioxidant power. The presented study is a continuation of our research on vegetables health-promoting capacity [9]. Some data presented in the paper related to the influence of thermal processing on TS require an explication.

Experimental

Materials

L-cysteine, 5,5'-dithiobis-(2-nitrobenzoic acid) (DTNB), gallic acid (GA), 2,4,6-tris(2-pirydyl)-s-triazine (TPTZ) were purchased from Sigma. 2,2-diphenyl-1-picrylhydrazyl (DPPH) was obtained from Aldrich. Folin-Ciocalteu phenol reagent was from Sigma-Aldrich. Other chemicals were obtained from POCh, Gliwice, Poland. All reagents used were of analytical grade.

Brussels sprouts material

Brussels sprouts (*Brassica oleracea* var. *gemmifera*) originating from traditional farming were purchased from local producers in south Poland. Randomized market sampling was applied. The average sample consisted of representative amounts of five individual samples from different farmers. The juices of two kinds of Brussels sprouts were studied:
I – stock Brussels sprouts, harvested in October *i.e.* the end of vegetation season in Poland;
II – Brussels sprouts after a 4-month storage at –4°C.

Methods

Preparation of plants juice

Brussels sprouts buds were sliced and pulped. The squeezed juices were filtered through gauze and the volumes were measured. Then the undissolved material was removed by centrifugation at 300 *g* for 10 min.

Thermal treatment of the vegetable juices

Brussels sprout juice was poured into 6 test-tubes. The juices were heated in a thermostat up to 22, 36, 50, 65, 80 and 95°C, respectively. When the defined temperature was reached the samples were maintained for 30 min and then they were taken out of the thermostat and cooled down to 22°C. The juices were 10-fold diluted with water before antioxidant activity determination. TS concentration was determined in the juices at natural concentration.

Spectrophotometric methods

All spectrophotometric measurements were done using Marcel-Media UV-Vis spectrophotometer. Absorbance was always recorded in a 1-cm quartz cuvette.

Determination of the antioxidant power by Folin-Ciocalteu method

Folin-Ciocalteu reagent contains a mixture of phosphomolybdic and phosphotungstic acid complexes. This method is considered to determine the contents of total phenolics [10]. However, the reagent reacts not only with phenolic but also with non-phenolic reducing substances *e.g.* hydrocarbon waxes, alkaloids, steroids and unsaturated compounds [11, 12] which form chromogen molybdenum blue that is spectrophotometrically detectable. Therefore, the absorbance corresponds rather to the total reducing capacity than to the total phenolic content.

Folin-Ciocalteu reagent (10-fold diluted with distilled water) was mixed with H_2O and a sample in the volume ratio 10:5:1, respectively. After a 3-minute incubation 1 cm^3 of 20% Na_2CO_3 was added and after further 15 min the absorbance was recorded at 730 nm [10].

The calibration curve was prepared with the use of gallic acid (GA) solutions in the range of concentrations 0.1-1.0 mg/100 cm^3. The absorbance was measured according to the procedure described above where GA solution was used instead of a juice sample.

Ferric reducing antioxidant power (FRAP)

The assay mixture consisted of 2.5 cm^3 300 mM acetate buffer, pH = 3.6; 0.25 cm^3 20 mM $FeCl_3$ and 0.25 cm^3 10 mM TPTZ in 40 mM HCl. The reaction was initiated by addition of 0.1 cm^3 of the juice 10-fold diluted with water. Absorbance at 593 nm was measured after a 10-minute incubation [13].

The calibration curve was prepared with the use of gallic acid solutions in the range of concentrations 0.025-0.300 mg/100 cm^3. Absorbance was measured according to the procedure described above.

Antiradical activity against DPPH

DPPH scavenging assay is commonly used for antioxidant activity determination in biological material [14, 15]. Here, this method was used for an aqueous-ethanolic system in volume ration 1:1.

Ethanolic solution (1 cm^3) of 0.3 mM DPPH was mixed with 1 cm^3 of water and 0.2 cm^3 of the juice (10-fold diluted with water). The decrease in absorbance in 1-cm quartz cuvette at 515 nm was measured in continuous mode for 20 min using a MARCEL MEDIA spectrophotometer.

Four different ways are used to recalculate the absorbance registered in DPPH method [16]:

1) reduction of absorbance (RA): $RA = A_{control} - A_{sample}$

2) scavenging activity percentage (AA%):
$AA\% = (A_{control} - A_{sample})/A_{control} \cdot 100\%$;

3) efficient concentration value (EC_{50}) defined as the concentration of the substrate that causes 50% loss of the DPPH activity;

4) antiradical efficiency (AE): $AE = 1/EC_{50} \cdot t_{EC50}$; where t_{EC50} value is the time needed to reach the steady state at EC_{50};

In this paper RA is applied as the simplest parameter of DPPH results description: $RA = A_{control} - A_{sample}$

where $A_{control}$ indicates absorbance of the blank which instead of the juice sample contained equal volume of water. The absorbances $A_{control}$ and A_{sample} were measured simultaneously.

The calibration curve was prepared with the use of gallic acid (GA) solutions in the range of concentrations 0.4-2.6 mg/100 cm^3. Absorbance was measured according the procedure described above.

Determination of thiosulfinate concentration

Thiosulfinate concentration was determined by applying spectrophotometric Han's method [17]. The method is based on the fact that one molecule of thiosulfinate reacts with two molecules of L-cysteine to form two molecules of S-alk(en)yl-mercaptocysteine. The decrease of L-cysteine content is measured spectrophotometrically at 412 nm in the form of 2-nitro-5-thiobenzoic acid (NTB) obtained in reaction L-cysteine with DTNB. The crude juice was mixed with 10 mM L-cysteine in the volume proportion 1:1 and incubated for 10 min at 26°C. Then 100 μM of the mixture was removed and added to 5 cm^3 of 0.15 mM DTNB. After a 10-minute incubation concentration of L-cysteine was determined indirectly by spectrophotometric measurement of the liberated NTB.

Results and discussion

Effect of storage on antioxidant quality and thiosulfinate content

Two kinds of Brussels sprouts were studied: the sprouts harvested in October (the end of vegetation season in Poland) and sprouts after a 4-month storage at –4°C. The

results of antioxidant capacity determined by the use of different methods and thiosulfinate (TS) content are summarized in Table 1.

The antioxidant capacity was expressed as gallic acid equivalent (GAE) in mg/100 cm^3 of the juice. It was shown that antioxidant ability determined with the use of Folin-Ciocalteu reagent and DPPH radical as well as TS content are higher by app. 20% for Brussels sprouts harvested in autumn than these determined after a 4-month storage. However, the FRAP method registered only app. 10% loss in GAE equivalent. The decrease of antioxidant ability and TS content during storage is caused by softening of the vegetable matrix and exposing substances to oxidation [18]. Phenolics (mostly responsible for the reaction with Folin-Ciocalteu reagent) and TS are compounds easy oxidative. The oxidation of phenolics results in creation respective quinones and TS forms polysulphides [8] which are not antioxidants.

Table 1. Thiosulfinate (TS) content and antioxidant capacity of Brussels sprouts juices in GAE mg/100 cm^3 determined by Folin-Ciocalteu, FRAP and DPPH methods

Kind of Brussels sprouts	Folin-Ciocalteu GAE mg/100 cm^3	FRAP GAE mg/100 cm^3	DPPH GAE mg/100 cm^3	TS mM
I	213±9	28±2	14.6±0.7	2.0±0.1
II	164±7	25±2	11.5±0.5	1.59±0.08

I – stock Brussels sprouts, harvested in October i.e. the end of vegetation season in Poland; II – Brussels sprouts after a 4-month storage at 4°C. All values are presented as mean ± SD for three replicates. The differences between I and II materials were statistically significant with $p < 0.05$ but FRAP ($p = 0.3$).

DPPH radical scavenging method standarization

DPPH is characterized as a stable free radical, soluble in organic solvents. DPPH radical scavenging method may be utilized in polar and nonpolar organic solvents and can be used to examine both hydrophilic and lipophilic antioxidants [19]. However, the method is commonly applied for determination antioxidant capacity in methanol or ethanol system and standardized for Trolox equivalent [20]. In this study, we used DPPH in ethanol/water system in volume ratio 1:1. The antioxidant sample was water diluted Brussels sprouts juice. The variant of the method utilized in this study simplifies the procedure, makes it safer (applying ethanol instead of methanol) as well as more economic. Moreover, measurement conditions better recreates the natural environment of the antioxidant action. Furthermore, gallic acid equivalent (GAE) is suggested for standardization because of its high effectiveness of DPPH scavenging [21].

However, comparing DPPH data with the other methods requires expression of RA in equivalents. The authors suggest to use gallic acid equivalent (GAE), the most commonly applied in antioxidant studies. A linear relationship between gallic acid concentration and RA in ethanol/water system used as a calibration curve with correlation coefficient $R^2 = 0.996$ proved that the used modified

procedure did not disturb the method (additional data). Moreover, the change of the DPPH solution absorbance caused by an antioxidant can be affected by influence of *e.g.* solvent, air or light [22]. To avoid this effect the simultaneous monitoring of DPPH solution and DPPH with the antioxidant was used.

Influence of thermal processing on antioxidant quality and thiosulfinate content

The antioxidant ability of Brussels sprouts juice was determined at 22, 36, 50, 65, 80 and 95°C after a 30-minute heating at respective temperature. The results obtained for Folin-Ciocalteu, FRAP and method with the use DPPH radical are presented in Figure 1. The decrease of the antioxidant capacity was in the range of 50% for Folin-Ciocalteu method until 80°C for the juice from fresh Brussels sprouts, the effect for the juice from Brussels sprouts storage was less spectacular, amounting to app. 20%. The lowering tendency was reversed at 95°C and antioxidant power was slightly regained. All observed trends are found to be statistically significant ($p < 0.05$). The trend was less clear for juice from Brussels sprouts after a 4-month storage ($p = 0.55$).The noticed effect might be result of the oxidation of some the antioxidants during storage. FRAP and DPPH scavenging methods indicated different trends showing that the antioxidant power was almost unchanged until 65°C while the processing at higher temperature increased of the antioxidant ability by app. 25% at $p < 0.05$. A few processes could cause this increasing antioxidant ability effect such as the liberation of antioxidant compounds due to the thermal destruction of cell walls and subcellular structures, thermal inactivation of oxidative enzymes, production of new antioxidants [23]. In contrast to change of the antioxidant activity, TS content decreased with increasing processing temperature until to the total loss (Figure 2). This proved that methylmethanethiosulfinate present in Brussels sprouts juices is a thermolabile compound not resistant for high temperature. The decay of TS was shown in both studied sorts of Brussels sprouts. This outcome is different from the data given by Olech et al. [9] which showed that TS content decreased only by 50% during heating. Since this inconsistency, the authors repeated the experiment several times, concluding that TS present in Brussels sprouts juices after a 30-minute heating at 95°C faded to zero.

In conclusion, the thermal processing of Brussels sprouts juice resulted in the total loss of TS, significant loss of the antioxidants reducing Folin-Ciocalteu reagent (mainly phenolics) and increase of the ability to Fe(III) reduce (revealed by FRAP method) and DPPH radical.

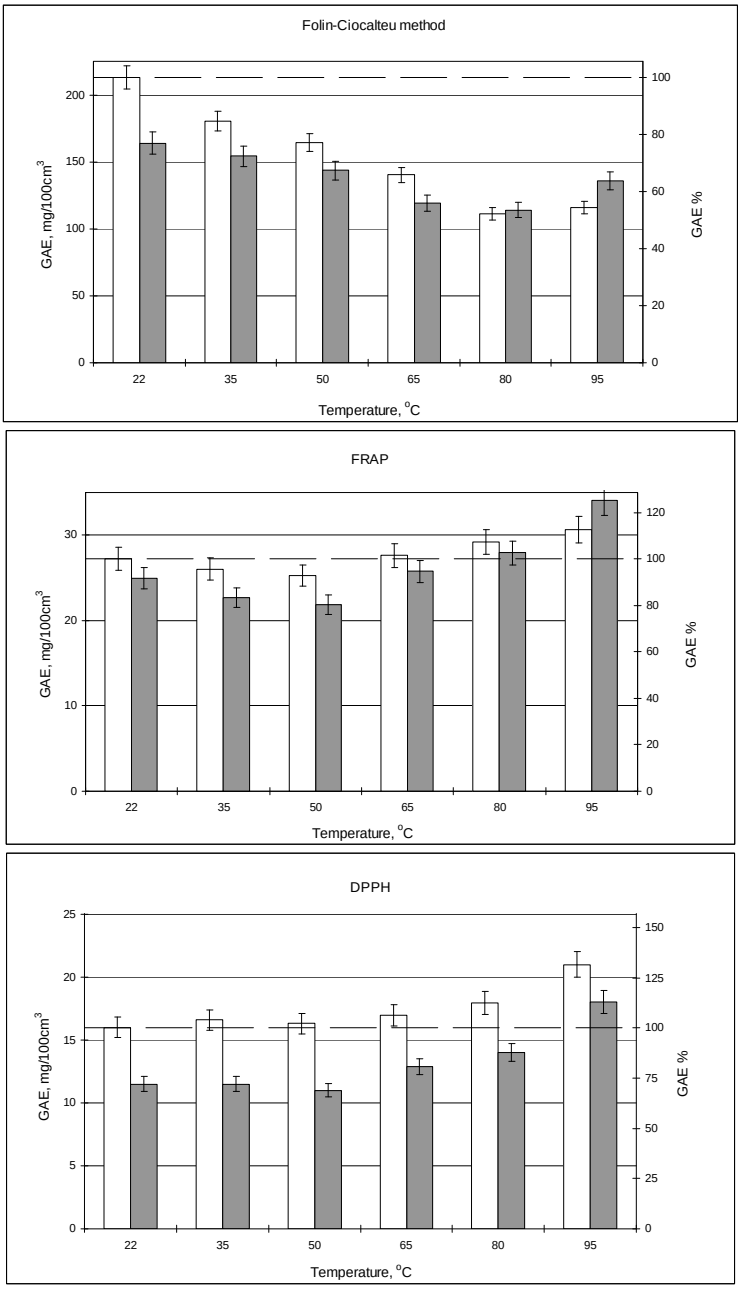

Figure 1. Dependence of the antioxidant power on the processing temperature determined by Folin-Ciocalteu, FRAP and DPPH radical scavenging methods. White bars relate to the juice from the stock Brussels sprouts, grey bars relate to the juice from Brussels sprouts after a 4-month storage at –4°C. All data are presented as mean ± SD, n = 3

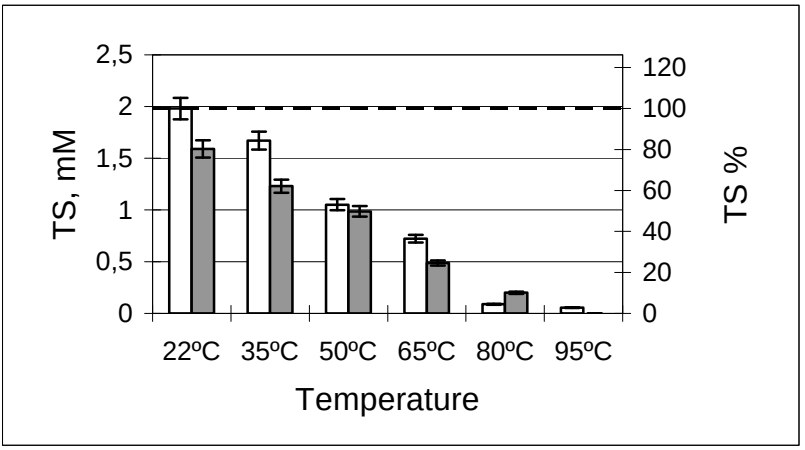

Figure 2. TS contents after thermal processing of Brussels sprout juice in different temperatures. White bars relate to the juice from the stock Brussels sprouts, grey bars relate to the juice from Brussels sprouts after a 4-month storage at –4ºC. All data are presented as mean ± SD, n = 3

Quantification and correlation between methods of antioxidant power determination

The antioxidant power was determined using 3 methods, each of them using reducing ability of the antioxidants against different media. Mo(VI) is reduced in Folin-Ciocalteu method, Fe(III) in FRAP method and radicals are scavenged in DPPH method. Moreover, each method required the different conditions *i.e*. Folin-Ciocalteu reagent is applied at alkaline pH = 10 which promotes phenolics to react, FRAP is used at acidic pH = 3.6; while alcohol is essential for solubility of DPPH. The highest value of GAE, app. 5 times higher than in the other methods, was obtained in Folin-Ciocalteu method. This indicates that phenolics are the main antioxidants present in Brussels sprouts juice. However, this conclusion could be misleading because each method is characterized by different mechanism, redox potential, pH and solvent. Therefore the results are hardly comparable. Interestingly, the GAE values determined by FRAP and DPPH methods are similar. Moreover, the relationships between GAE values determined by FRAP and DPPH methods are represented by the linear relationships with the high correlation coefficients (Figure 3). This could point to the common antioxidants being active in both methods. On the other hand, the relationships between GAE for Folin-Ciocalteu and FRAP as well as Folin-Ciocalteu and DPPH methods are characterized by low correlation coefficients (below 0.5). This indicates that dominant antioxidants revealed by Folin-Ciocalteu method are different than those showed by FRAP and DPPH procedures. The reason can be significantly higher pH applied for Folin-Ciocalteu method.

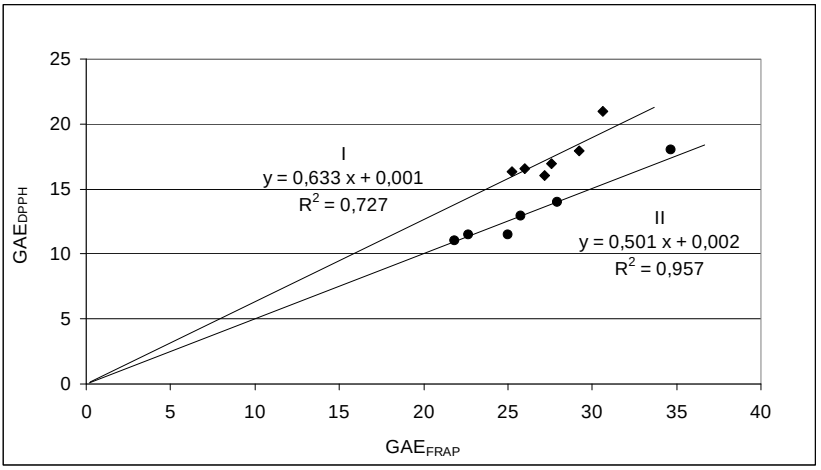

Figure 3. Linear relationship between GAE values obtained by FRAP method and GAE obtained by the use of DPPH radical. Square symbols relate to the juice from the stock Brussels sprouts, circles relate to the juice from Brussels sprouts after a 4-month storage at –4°C

Some authors considered TS as the antioxidants [24]. Our studies did not confirm this thesis. The processing in high temperature resulted in the total TS loss (Figure 2) but the change in the TS concentration did not reflect the change in antioxidant power (Figure 1). However, this observation does not exclude entirely TS antioxidant abilities. Abundance of antioxidants in Brussels sprouts juice can obscure TS impact on resultant antioxidant power. Therefore the total loss of TS can be negligible among other Brussels sprouts antioxidants.

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