POLISH JOURNAL OF FOOD AND NUTRITION SCIENCES

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Pol. J. Food Nutr. Sci.
2007, Vol. 57, No. 2(A), pp. 201-206

INFLUENCE OF STERILIZATION PROCESS ON ANTIOXIDATIVE PROPERTIES OF BROAD BEAN SEEDS

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Key words. broad bean, antioxidant activity, processing, sterilization, proteins, polyphenols

Changes of selected antioxidants content and activity in broad bean seeds upon sterilization process and storage of cans were investigated in the study. Loss of total nitrogen, its solubility and non-protein nitrogen content was observed as well as loss of total polyphenols content and their antioxidatively important group – condensed tannins. Main changes in protein fractions were observed on electrophoretic patterns in low molecular weight range, however a gain of high molecular weight polymer (500 kDa) participation was shown by Size-Exclusion HPLC. Phenolic compounds extracted with 70% acetone exhibited much higher antiradical activity then nitrogen compounds extracted with water. A decrease of antioxidant activity of the extracts and its appearance in drain was confirmed in most cases after processing and storage.

INTRODUCTION

During the last several years the attitude toward food has been changing rapidly. Additionally to its nutrition and sensory properties a third function is becoming increasingly apparent. Due to a great number of compounds and chemical and biological complexities food may exhibit a protective action and modulate physiological systems such as the immune, endocrine, nervous and digestive system.

Phytochemicals present in various natural foods such as antioxidants, fibers and other bioactive compounds exhibit beneficial health effects as well as affect stability and shelf-life of food. Among numerous phytochemicals attention is paid to flavonoids and antioxidants. Quercetin and other flavonoids show anticancerogenic and antiproliferative effects [Hollman *et al.*, 1996]. Moreover, most flavonoids are effective free radical scavengers. Antioxidant, anticancer and antimutagenic substances are found in many foods, but tea, spices, herbs and allium plants deserve special attention.

Legumes belong to that group of food raw materials, which besides nutrient important compounds provide bioactive substances such as isoflavones [Braakman, 2003; Hertog *et al.*, 1992] and antioxidants [Troszynska & Kubicka, 2001]. The antioxidant activity is due to phenolic compounds [Druzynska, 2002] and proteins [Wolosiak & Klepacka, 2002], in which some amino acid derivatives may act as natural antioxidants. Such antioxidant activity is attributed to ε-N-pyrrolylmorleucine [Zamora *et al.*, 1999]. Legumes are considered as having medium concentration of flavonoids, and in broad beans their content is below 50 mg/kg [Hertog *et al.*, 1992].

The beneficial health effect seems to be achieved only through consumption of foods naturally rich in bioactive compounds. This observation is very important because processing and storage may change biological activity of food.

Generally it is thought that processing, especially heat treatment, reduces antioxidant and bioactive properties of food. However, the ways the processing affects those properties can vary from “no effect” through “decrease” to even “improvement”. It has been shown that moderate heating increases bioavailability of β-carotene [Nicoli *et al.*, 1999]. On the other hand ascorbic acid and polyphenols are consumed in the Maillard reactions and the antioxidant activity of thermally treated fruits and vegetables is depelted. Polyphenols in an intermediate state of oxidation are very active antioxidants, while in chemically or enzymatically oxidized state show decreased antioxidant properties. It was recorded that antioxidant properties of tea extracts bottled and pasteurized increased during a 30 day storage [Manzocco *et al.*, 1998].

Processing of food can lead to formation of new compounds. The Maillard reaction products are generally known as strong antioxidants, and the antioxidation properties are mainly attributed to the high molecular weight brown compounds [Nicoli *et al.*, 1999]. Reactions between natural antioxidants and between antioxidants and oxidation products may form compounds with modified antioxidant properties. Processing can enhance or promote these reactions.

Heat sterilization is an unit operation in which food is heated to temperatures exceeding 100°C for a sufficiently long time in order to destroy microorganisms and to cook food. Sterilization in cans, due to severe heat treatment causes

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changes in sensory and nutritional quality of food. During sterilization chlorophyll is converted to pheophitin, carotenoids are isomerized from 5.5-epoxides to 5.8-epoxides, anthocyanins are degraded to brown pigments and oxymioglobin is converted to brown metmyoglobin [Fellows, 1988]. Maillard reactions as well as degredation or recombinant reactions form hundreds different compounds, which modify original flavor and are responsible for formation of typical cooked taste and aroma. Denaturation of proteins and loss of water binding properties are responsible for changes in texture and viscosity of sterilized food. Substantial changes in nutritional quality of food are also recorded. Content of essential amino acids and vitamins is reduced and strongly depends on time-temperature schedule of sterilization process.

Broad bean seeds are rich in protein and other nutrition important compounds [Kmiecik & Lisiewska, 1994; Gebczynski, 1995]. They are commercially canned, however the effect of sterilization on broad bean seeds nutritional quality and, especially antioxidant activity is not well recognized. Taking into account that sterilization causes many changes in chemical composition of processed product it can be expected that that process may equally change antioxidant activity.

The aim of this work was to investigate the antioxidant properties of raw broad bean seeds and the influence of sterilization process on these properties.

MATERIAL AND METHODS

For the investigations broad bean *(Vicia faba)* seeds variety Windsor Biały of proper technological maturity were used. Seeds were blanched in water at 92°C for 5 min. Immediately after blanching seeds were packed into 400 g cans. Solution of 1.3% NaCl was used and the ratio between seeds and solution was 6:4 ww. Sterilization was done in the autoclave Rotorzwerg (Stock). Temperature of heating medium and the contents of the cans was measured with thermometers SSA 12050 G 7000 TS connected to four channel recorder CTF 9004 (Ellab). The sterilization value Fo was automatically calculated. Sterilization was done according to the formula:

$$\frac{10-18-16}{120.7}; \quad Fo = 13.52$$

Changes of temperature and sterilization value Fo are shown in Figure 1.

The investigations were performed on raw material and after sterilization followed by 1 year of storage, for which water and 70% acetone extracts were prepared. To accomplish this the raw seeds or after separation from the drain were ground in a laboratory mill (Retsch Grindomix GM200), then mixed with a proper solvent in a ratio 1:10 (w/v) and shaken for 2 h at room temperature.

In the ground preparations total protein, dry matter and ash content were determined, while in the extracts the content of compounds exhibiting antioxidative properties. In water extracts the protein content and non-protein nitrogen content were determined, in acetone extracts total polyphenols content – expressed as gallic acid [Singleton & Rossi, 1965] and condensed tannins content – expressed as (+)catechin [Price, 1978].

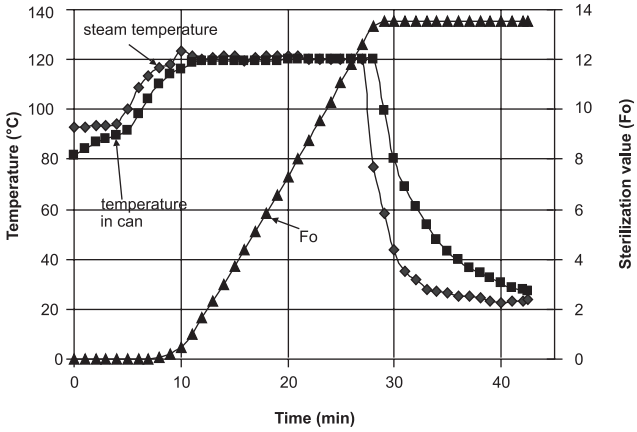

FIGURE 1. Changes of steam temperature, temperature in can and sterilization value during processing of broad bean seeds.

To characterize the proteins present in water extracts they were separated by HPLC method on Supradex 200 HR 10/30 column. The detection was lead at 280 nm (Shimadzu UV SPD-6A detector). Mobile phase (50 mmol/L Na-phosphate buffer pH 7 + 0.15 mol/L NaCl) was used at the flow of 0.4 mL/min. Peaks were integrated using Shimadzu C-R6A integrator. The column was calibrated by the molecular weight markers (Pierce): cytochrom C (12.5 kDa), chymotrypsynogen (25 kDa), egg albumin (45 kDa), bovine serum albumin (67 kDa), catalase (158 kDa), ferritin (240 kDa) and Blue Dextran (2000 kDa). The extracts (100 μl) were injected after filtration by syringe filters (pore size 45 μm). Protein fractions were also separated by electrophoresis (SDS-PAGE) on 12% polyacrylamide gels.

Also chelating ability investigations towards Fe(II) ions were run in water as well as acetone extracts by the method of Lai *et al.* [2001]. Chelating was calculated basing on the absorbance of Fe-ferrozine complex decrease (=562 nm).

Broad bean extracts antiradical activity was determined towards $ABTS^{\bullet+}$ radical cations [Re *et al.*, 1999] and $DPPH^{\bullet}$ stable radicals [Yen & Chen, 1995] expressing the results as mg of Trolox (antioxidant standard used for calibration curve) per 1g of sample and 1g of dry matter. Antioxidative activity was also measured as an ability to inhibit linoleic acid emulsion oxidation in autoxidation reaction catalysed by hemoglobin [Kuo *et al.*, 1999] and enzymatic reaction catalysed by lipoxigenase. In both cases the amount of hydroperoxides was measured applying spectrophotometric (λ=480 nm) ferric thiocyanate method after the reactions were stopped by 0.5% HCl in ethanol addition. The results were expressed as percent of oxidation's inhibition using control as a marker of the process scope without antioxidative compounds.

RESULTS AND DISCUSION

Basing on the performed investigations it was observed that in case of fresh material total nitrogen content equaled 5.3% of dry matter, while after sterilization process 4.5% d.m. (Table 1). In water extracts derived from raw material the amounts of soluble nitrogen and non-protein nitrogen were determined on the levels of 27.7% and 25.2% of total nitrogen, respectively. After the sterilization process the content of

TABLE 1. The experimental material characterisation.

Sample	Total N (g/100 g d.m.)	Soluble N (g/100 g total N)	Non-protein N (g/100 g total N)	Total polyphenols (mg/100g d.m.)	Condensed tannins (mg/100g d.m.)	Chelating ability (water soluble) (μmol Fe/g d.m.)	Chelating ability (acetone soluble) (μmol Fe/g d.m.)
Raw material	5.33 (±0.07)	27.75 (±0.46)	25.23 (±0.53)	735 (±1.23)	330 (±1.1)	9.36 (±0.02)	7.81 (±0.02)
Sterilized material	4.51 (±0.09)	25.66 (±0.76)	23.68 (±0.00)	430 (±1.24)	78 (±1.92)	11.01 (±0.05)	7.52 (±0.02)
Drain	11.88 (±0.25)	n.d.*	45.22 (±1.15)	n.d.	11 (±0.002)	29.11 (±0.06)	n.d.

* not determined

TABLE 2. Antiradical activities of the extracts investigated.

Sample	Activity towards $ABTS^{\bullet+}$ (mg Trolox/g sample)	Activity towards $ABTS^{\bullet+}$ (mg Trolox/g d.m.)	Activity towards $DPPH^{\bullet}$ (mg Trolox/g sample)	Activity towards $DPPH^{\bullet}$ (mg Trolox/g d.m.)
Water extract of raw material	8.78 (±0.20)	41.79 (±0.93)	0.25 (±0.01)	1.19 (±0.30)
Acetone extract of raw material	13.88 (±0.23)	66.12 (±1.08)	5.18 (±0.06)	24.64 (±0.26)
Water extract of canned material	5.16 (±0.03)	30.36 (±0.19)	0.24 (±0.01)	1.42 (±0.03)
Acetone extract of canned material	6.05 (±0.37)	35.59 (±2.15)	2.09 (±0.02)	12.31 (±0.16)
Drain	3.18 (±0.02)	158.89 (±0.98)	0.48 (±0.03)	23.88 (±1.12)

soluble proteins and non-protein nitrogen slightly decreased comparing to raw material and equaled 25.7% and 23.8% t.n., respectively. The drain obtained from the cans contained 11.9% d.m. nitrogen in which about 45% was constituted by non-protein nitrogen. Water extracts prepared from raw material exhibited an ability to chelate Fe ions on the level of 9 μmol/L Fe/g d.m. In case of the extracts obtained after the process an increase of chelating ability to about 11 μmol/L Fe/g d.m. was observed and dry matter constituents in the drain exhibited even better chelating ability – 29 μmol/L Fe/g. This was probably due to the changes in the material structure by heat which may lead to a better extractability of active compounds; also protein denaturation and exposure of internal amino acids is worth mentioning.

Total polyphenols content decreased after sterilization (from 730 do 430 mg/100g d.m.). A decrease of condensed tannins content after heat treatment (from 330 to 78 mg/100 g d.m.) was noted as well. Similar tendencies obtained also Wilska-Jeszka & Stasiak [1994]. Transition ions chelating properties have a big influence on antioxidative properties of phenolic compounds, therefore in the study their ability to bind Fe(II) ions determination was also performed. On the contrary to water extracted substances, fresh broad bean acetone extract exhibited very similar, but better chelating properties than the one obtained after sterilization (7.8 and 7.5 μmol Fe/g d.m., respectively). One of the reasons for this may be a higher content of condensed tannins in raw material extract. Some authors indicate that these polyphenols have the strongest properties of transition ions binding [Sanchez-Moreno *et al.*, 1998].

Basing on the electrophoretic patterns obtained (Figure 2) it was stated that among the raw broad bean proteins (1A) dominant were the fractions of 47 kDa, 32 kDa and 12 kDa, of which two first respond to the molecular weights of 11S globulin components [Gueguen, 1991]. After sterilization (3B) some changes occurred in low weight fractions, in which 15 kDa was the dominant one. In the extracts the main seed protein fractions were absent whereas a fraction of 10 kDa molecular weight was found. In the drain fractions' participation was more diverse comparing to the extracts, but also the one of about 11 kDa was dominating. Gel chromatography separations (Figure 3) proved that in the extracts investigated the fractions of molecular weight lower than 10 kDa dominate and their highest participation was found in the raw seeds extracts (98%). An increase of 500 kDa fraction participation from 0.1% in raw seeds extract to 2.3% in sterilized seeds extract was also observed. A reverse phenomenon was stated by Carbonaro *et al*. [1997], who examining dry broad bean seeds found a decrease of high molecular weight fractions in water extracts after cooking.

Sterilization process caused a significant decrease of antiradical activity (by approx. 60%) towards $ABTS^{\bullet+}$ and $DPPH^{\bullet}$ radicals of the acetone extracts (Table 2). The activity of water extracts towards $ABTS^{\bullet+}$ radicals decreased in lower, but comparable degree (by approx. 40%). A confirmation of this antioxidative compounds' changes tendency may be also found in the results of the total antiradical activity obtained for the ground material homogenized with water (without extraction), which decreased from 24.5 to 10.6 mg Trolox/g of sample (that is almost by 60%) after sterilization process, which stands in agreement with the data published by Hunter & Fletcher [2002], who found approx. 60% decrease of total water soluble antioxidant activity in canned peas and over 70% in canned spinach. No changes in the ability of water extracts

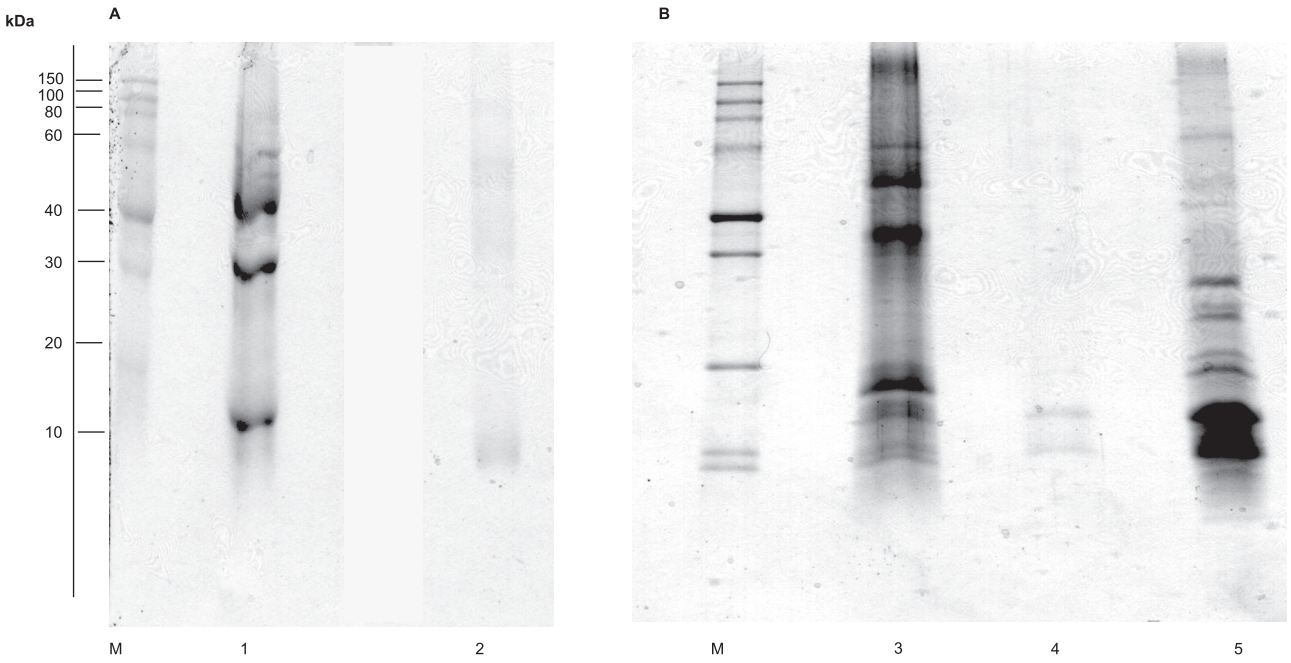

FIGURE 2. Electrophoretic patterns of the proteins: total fractions from raw (1A) and sterilized (3B) seeds, water extracts from raw (2A) and sterilized (4B) seeds and the drain (5B); M=molecular weight marker.

to deactivate DPPH$^{\bullet}$ radicals was observed in our investigations, however in this case the activity was very low (Table 2).

The results described above concern the activities expressed in mg Trolox per 1 g of sample. Taking into consideration the change of dry matter content the results were also expressed per 1 g of dry matter. A little lower decrease of the antiradical activity of the compounds extracted after sterilization was stated (from 30 to 50%) then it was arising from the values calculated for the whole sample and in case of the water extracts action towards DPPH$^{\bullet}$ radicals even approx. 20% gain was observed. Similar results were gathered by Turkmen *et al.* [2005], who found a little decrease (16%) of the boiled peas activity calculated basing on the dry matter and a big gain of activity for green beans (by 60%).

Basing on the investigations conducted it was stated that the deterioration of antioxidant activity of broad bean after sterilization and storage is not only connected with the changes undergoing upon the heat treatment of the seeds components, but also with their significant loss to the drain during the whole period of storage. The activity of constituents present in the drain towards ABTS$^{\bullet+}$ radicals equaled approx. 50% of the values obtained for water and acetone extracts and in case of DPPH$^{\bullet}$ radicals deactivation the drain activity was even higher than water extracts activity. The results for the drain expressed per dry matter show that the compounds present there were several times more active towards the radicals applied than the extracted from the processed seeds.

TABLE 3. Antioxidant activities of the extracts investigated.

Sample	Activity against peroxides in hemoglobin-catalysed process (%)	Activity against peroxides in lipoxigenase-catalysed process (%)
Water extract of raw material	17.6 (±1.6)	13.9 (±4.7)
Acetone extract of raw material	100 (±0.5)	53.5 (±5.3)
Water extract of canned material	18.6 (±2.15)	21.2 (±4.0)
Acetone extract of canned material	39.2 (±1.27)	35.3 (±3.3)
Drain	15.0 (±1.25)	9.8 (±2.2)

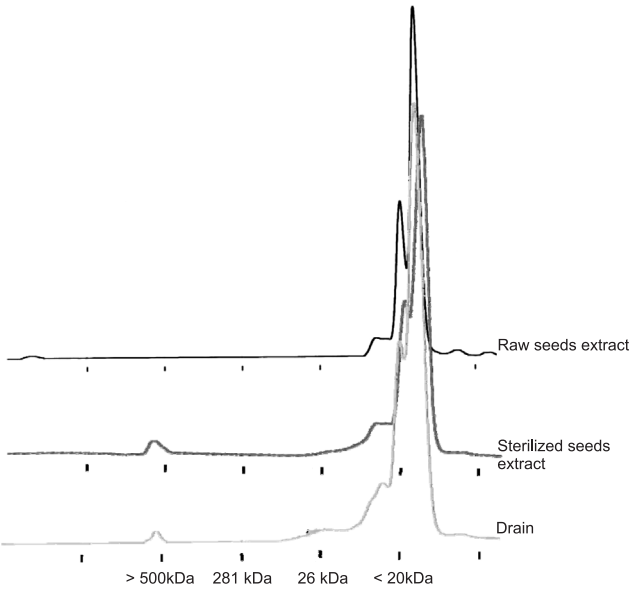

FIGURE 3. Gel filtration chromatograms of protein fractions in water extracts from the broad bean seeds before and after processing and in the drain.

Acetone extracts exhibited much better antioxidant activity towards linoleic acid hydroperoxides comparing to those obtained by water (Table 3). The application of sterilization process caused an important deterioration of the linoleic acid autoxidation inhibiting ability (by over 60%) for the substances extracted with acetone, which activity after the raw material extraction reached 100%. Also approx. 35% decrease of their activity in lipoxygenase-catalysed reaction was noted.

No distinct change of the water extracts' antioxidant activity after broad bean thermal treatment in linoleic acid autoxidation was stated (5% gain), while these extracts performance in the reaction catalysed by lipoxygenase underwent a clear improvement (50% increase). In both oxidation systems applied the activity was proved for drain as well, 15 and 10%, respectively.

In the study there were three correlations found: activity of the water extract towards $ABTS^{\bullet+}$ radicals (ABTS) was significantly dependent upon the non-protein nitrogen content (N) at 95% confidence level: ABTS = -109 + 5.9*N (r=0.9999), which shows that well soluble and better surface exposed non-protein nitrogen (on the contrary to many proteins) is an important factor of water-extracted active substances; also the acetone extract activity in autoxidation process (LOOH) was correlated with the tannins content (T) at 90% confidence level: LOOH = 15 + 0.26*T (r=0.997), which shows their important role among polyphenols as antioxidants. The activities in autoxidation process (LOOH) were also correlated with the activities in lipoxigenase-catalysed system (LOX): LOOH = e^(2 + 0.04*LOX) at 99% confidence level (r=0.988). However, especially first two calculations were based on very few measuring points, so these correlations should be rather treated as some indicators.

CONCLUSIONS

1. Even though a loss of both water extracted (nitrogen compounds) and acetone extracted (phenolic compounds) constituents was observed upon sterilization and storage of broad bean seeds, the Fe chelating ability of the extracts underwent gain or no significant change.

2. Antioxidant activity of the extract towards $ABTS^{\bullet+}$ radical cations was much better comparing with $DPPH^{\bullet}$ radicals while the activities in the linoleic acid oxidation process catalysed by hemoglobin and lipoxigenase were usually balanced.

3. Dry matter constituents partly lost their activity in most cases and very active compounds appeared in the drain, however some gain of active compounds was also noted in the extracts, probably due to improvement of extractability and modification upon heating.

ACKNOWLEDGEMENTS

Research work funded from the means of the Minister of Science in 2004-2006 as a solicited project.

REFERENCES

1. Braakman L., Obstacles for antioxidants. Food Eng. Ingredients, 2003, 28, 46-49.
2. Carbonaro M., Cappelloni M., Nicoli S., Lucarini M., Carnovale E., Solubility-digestibility relationship of legume proteins. J. Agric. Food Chem., 1997, 45, 3387-3394.
3. Druzynska B., Polyphenolic compounds of bean seed coats (*Phaseolus vulgaris* L.) and their antioxidant properties. Pol. J. Food Nutr. Sci., 2002, 11/52(4), 35-39.
4. Fellows, P. Food Processing Technology. Principles and Practice. 1988, Ellis Horwood, Chichester.
5. Gebczynski P., Effect of maturation degree of seeds on nutritve value and technological usability of broad bean. Zesz. Naukowe Akademii Rolniczej w Krakowie, 1995, 301, 43-53.
6. Gueguen J., Developments in Food Proteins-6. 1991, Elsevier Applied Science, London and New York.
7. Hertog M.G.L., Hollman P.C.H., Katan M.B., Content of potentially anticarcinogenic flavonoids of 28 vegetables and 9 fruits commonly consumed in the Netherlands. J. Agric. Food Chem., 1992, 40, 2379-2383.
8. Hollman P.C.H., Hertog M.G.L., Katan M.B., Analysis and health effects of flavonoids. Food Chem., 1996, 57(1), 43-46.
9. Hunter K.J., Fletcher J.M., The antioxidant activity and composition of fresh, frozen, jarred and canned vegetables. Inn. Food Sci. Emer. Technol., 2002, 3, 399-406.
10. Kmiecik W., Lisiewska Z., Chemical composition of broad bean seeds depending on cultivar and degree of seed maturation. Folia Horticulturae, 1994, X(1), 23-30.
11. Kuo J.-M., Yeh D.-B., Pan B.S., Rapid photometric assay evaluating antioxidative activity in edible plant material. J. Agric. Food Chem., 1999, 47, 3206-3209.
12. Lai L.S., Chou S.T., Chao W.W., Studies on the antioxidative activities of Hsian-tsao leaf gum J. Agric. Food Chem., 2001, 49, 963-968.
13. Manzocco L., Anese M., Nicoli, M.C., Antioxidant properties of tea extracts as affected by processing. Lebens. Wiss. Technol., 1998, 31, 694-698.
14. Nicoli M.C., Anese M., Parpinel M., Influence of processing on the antioxidant properties of fruit and vegetables. Trends Food Sci. Technol., 1999, 10, 94-100.
15. Price M.L., Van Scoyoc S., Butler L.G., A critical evaluation of the vanillin reaction as an assay for tannin in sorghum grain. J. Agric. Food Chem., 1978, 26, 1214-1218.
16. Re R., Pellegrini N., Proteggente A., Pannala A., Yang M., Rice-Evans C., Antioxidant activity applying an improved ABTS radical cation decolorization assay. Free Rad. Biol.Med., 1999, 26, 1231-1237.
17. Sanchez-Moreno C., Larrauri J.A., Saura-Calixto F., A procedure to measure the antiradical efficiency of polyphenols. J. Sci. Food Agric., 1998, 76, 270-276.
18. Singleton V.L., Rossi J.A., Colorimetry of total phenolics with phosphomolybdic-phosphotungstic acid reagents. Am. J. Enology Viticulture, 1965, 16, 144-158.
19. Troszynska A., Kubicka E., Superoxide scavenging activity of seed coat extracts from legume seeds. Pol. J. Food Nutr. Sci., 2001, 10/51(4), 55-59.
20. Terkmen N., Sari F., Sedat Velioglu, Y., The effect of cooking methods on total phenolics and antioxidant activity of selected green vegetables. Food Chem., 2005, 93, 713-718.
21. Wilska-Jeszka J., Stasiak A., Polyphenol compounds in grain legumes. bioactive substances in food of plant origin. Materials of the International Euro Food Tox IV Conference, 22-24 September 1996, Olsztyn, Poland, 1, 126-130.

22. Wolosiak R., Klepacka M., Antioxidative properties of albumins in enzymatically catalyzed model systems. Electronic Journal of Polish Agricultural Universities, 2002, 5(1), [http://www.ejpau.media.pl/series/volume5/issue1/food/art-05.html].
23. Yen G-C., Chen H-Y., Antioxidant activity of various tea extracts in relation to their antimutagenicity. J. Agric. Food Chem., 1995, 43, 27-32.
24. Zamora R., Alaiz M., Hidalgo F.J., Determination of ε-N-pyrrolylmorleucine in fresh food products. J. Agric. Food Chem., 1999, 47, 1942-1947.

WPŁYW PROCESU STERYLIZACJI NA ZMIANY WŁAŚCIWOŚCI ANTYOKSYDACYJNYCH NASION BOBU

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W pracy badano zmiany zawartości i aktywności wybranych przeciwutleniaczy z nasion bobu pod wpływem procesu sterylizacji i przechowywania otrzymanych konserw. Obserwowano spadek zawartości azotu ogółem, jego rozpuszczalności i zawartości azotu niebiałkowego, jak również spadek zawartości polifenoli ogółem oraz ich ważnej w aspekcie działania przeciwutleniającego grupy – skondensowanych tanin. Główne zmiany wśród frakcji białkowych stwierdzono przy pomocy rozdziałów elektroforetycznych w zakresie białek drobnocząsteczkowych, jednak także wzrost udziału frakcji wysokocząsteczkowej (500 kDa) wykazano przy pomocy SE-HPLC. Związki fenolowe ekstrahowane 70% acetonem wykazywały znacznie większą aktywność przeciwrodnikową, niż związki aminowe ekstrahowane wodą. W większości przypadków po przetwarzaniu i przechowywaniu nasion stwierdzono spadek aktywności przeciwutleniającej ekstraktów i pojawienie się jej w zalewie.