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BIOLOGICALLY ACTIVE SUBSTANCES IN THE BROAD BEAN GREEN SEEDS AFTER STORAGE IN THE PODS

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Abstract. Broad bean seeds, with a high nutritional value at green maturity stage, are used for direct human consumption and in food processing. However broad bean green seeds had as a very shortly time to use because the green seeds very quickly lost the white-green fresh colour. The aim of the study was to evaluate the content of biologically active substances in fresh broad bean seeds immediately after the harvest and seeds from pods stored for a week under low temperature conditions. Experimental material consisted of fresh, green broad bean seeds of Bachus, Basta, R-366/1, Bolero, Jankiel Biały, and Windsor Biały cultivars. Broad bean seeds were sown in the second decade of April at 60 × 10 cm spacing, 100 seeds per plot of 6.0 m^2 area. Broad bean pods were harvested once at the stage of technological maturity of seeds in the second and third decade of July. Marketable seed samples of 500 g were collected for laboratory tests. At the same time, immediately after the harvest, samples of 2 kg marketable broad bean pods of each cultivar were collected and storage within 7 days at 1–4°C. The contents of L-ascorbic acid, chlorophyll (a + b), flavonoids (QE), free phenolic acids sum (CAE), and dry matter were determined in broad bean seeds directly after the harvest as well as in the seeds from the pods stored for 7 days. In the results of the investigation indicate that green faba bean seeds from the storage pods had a good white-green colour and marketable quality, however the seeds had the significantly lowest content of L-ascorbic acid and chlorophyll (a + b). The content of selected biologically active substances in the seeds after storage in the pods indicates a significant difference in dependence on cultivar.

Key words: *Vicia faba* L. var. *major*, flavonoids, phenolic acids, chlorophyll, L-ascorbic acid

INTRODUCTION

Fruits and vegetables are a source of valuable nutrients and biologically active substances, which play a very important role in the normal human diet, show pro-health and antioxidant properties, and have an impact on functioning and health of our organism

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[Hertog 1996, Małolepsza and Urbanek 2000, Troszyńska et al. 2000, Wolski and Dyduch 2000, Lipecki and Libik 2003, Michniak et al. 2004, Nowak 2004, Budryn and Nebesny 2006, Sałata and Buczkowska 2007, Najda and Buczkowska 2013, Najda and Łabuda 2013, Parus 2013, Kałędkiewicz and Lange 2013].

The most important biologically active substances, that are common in vegetables and fruits and having antioxidant properties, are plant-origin secondary metabolites such as polyphenolic compounds (phenolic acids, flavonoids), plant pigments, and glucosinolates. Among vitamins, the most important ones showing the antioxidant properties include vitamin E (tocopherol), vitamin C (ascorbic acid), and pro-vitamin A (retinol). Deficiency of these vitamins in a diet increases the body's susceptibility to the adverse effects of free radicals [Chu et al. 2000, Hunter and Fletcher 2002, Bahorun et al. 2004, Sembratowicz and Czech 2005, Bołonkowska et al. 2011].

Broad bean (*Vicia faba* L.), like any other plant in the *Fabaceae* family, is important in growing and feeding people, it is one of the foods with health-promoting effects due to the presence of protein, fiber, oligosaccharides, mineral constituents and vitamins, including vitamin B, in seeds. Antioxidant properties of biologically active substances, especially polyphenolic compounds found in seeds and other parts of broad bean plant, were also demonstrated [Hertog et al. 1992, Kmiecik et al. 2000, Troszyńska et al. 2000, Macarulla et al. 2001, Drużyńska and Jeżak 2007, Al-Numair 2009, Crépon et al. 2010, Duc et al. 2010, Köpke and Nemecek 2010, Chaieb et al. 2011, Giménez et al. 2012].

In Europe, broad bean is grown mainly for fresh, immature seeds intended for direct consumption or for processing (freezing, canning). However, the broad bean seeds at the stage of green technological maturity, have a short shelf-life and use in processing, lose their quality due to rapid darkening as a result of the oxidation processes.

The aim of the study was to evaluate the content of selected biologically active substances in fresh seeds of six broad bean cultivars immediately after harvesting and in seeds from the pods and stored for a week under low temperature conditions (1–4°C).

MATERIAL AND METHODS

The field studies were carried out in 2008–2009 at the Felin Experimental Farm of the University of Life Sciences in Lublin (51°14'N, 22°18'E, and altitude of 215 m). Experimental material consisted of fresh, green broad bean seeds of Bachus, Basta, R-366/1, Bolero, Jankiel Biały, and Windsor Biały cultivars. Broad bean was grown in lessive soil developed from the loess formation. Fertilization was based on results of the soil analysis. Broad bean seeds were sown in the second decade of April at 60 × 10 cm spacing (16.6 plants m^{-2}), 100 seeds per plot of 6.0 m^2 area. The experiment was established by means of randomized blocks in four replicates. During the vegetation period, plant protection treatment according to recommendations for broad bean, were made.

Broad bean pods were harvested once at the stage of technological maturity of seeds (75% of developed pods per plant), which occurred in the second and third decade of July. Marketable pods were manually de-husked, and seed samples of 500 g were collected for laboratory tests. At the same time, immediately after the harvest, samples of 2 kg marketable broad bean pods of each cultivar were collected and placed in plastic

bags in a refrigerator at 1–4°C temperature and relative humidity of 85–90% for the storage within 7 days. The experiment was carried out in triplicate.

In the seeds directly at harvest and in the seeds after storage in pods were determined the following selected biologically active substances.

L–ascorbic acid content. Determinations of L–ascorbic acid contents were performed according to Roe with Ewelin's modifications [Korenman 1973]. Aliquots of 2.000 g (in triplicate) of fresh, green broad beans were homogenized in a mortar with small amount of 2% water solution of oxalic acid. Homogenizates were then filtered through cotton into the 100 ml volumetric flask washing the precipitate with oxalic acid solution and adjusting volume to 100 ml. Aliquots of 2 ml of filtrates were taken from flasks and 10 ml of 2,6-dichloroindophenol solution was added. The absorbance measurements were made using spectrophotometer Hitachi U-2900 spectrophotometer (Canberra Packard) at the wavelength λ = 520 nm using 1 cm optical path length cuvettes in relation to blank sample (2 ml of 2% oxalic acid solution + 10 ml of 2,6-dichloroindophenole solution + several crystals of standard L–ascorbic acid to decolorize). Then several crystals of standard L-ascorbic acid was added in order to completely decolorize the solution and another absorbance measurement was made to eliminate the errors due to turbidity and own color of the sample and the pigment. Content of L-ascorbic acid in test samples was determined on a base of calibration curve (A = f(c)) prepared for different concentrations of L-ascorbic acid standards.

Chlorophyll a and b contents. Determinations of chlorophyll a, b and sum of a and b were carried out by means of spectrophotometry according to Mac Kinney [Charłampowicz 1966]. Aliquots of 2.000 g (in triplicate) of fresh, green broad beans were homogenized in a mortar with small amount of 80% water with acetone mixture. The product was then filtered into the 100 ml volumetric flask washing the precipitate with 80% water solution of acetone and adjusting volume to 100 ml. Aliquots of 2 ml of filtrates were taken from flasks and absorbance of light passing through the solution was measured in relation to blank sample (80% water solution of acetone) at wavelengths λ = 663 nm and λ = 645 nm. The total content of chlorophyll (C) was calculated in $mg \cdot g^{-1}$ according to the formula:

$$C = \left(20.2E_{645} + 8.02E_{663}\right) \cdot \frac{a}{100\,\mathrm{g}}$$

Content of chlorophyll a was calculated in $mg \cdot g^{-1}$ according to the formula:

$$C_a = 20.2E_{645} \cdot \frac{a}{100\,\mathrm{g}}$$

Content of chlorophyll b was calculated in $mg \cdot g^{-1}$ according to the formula:

$$C_b = 8.02E_{663} \cdot \frac{a}{100\,\mathrm{g}}$$

where:

a– volume of the extract,

g – weight of the sample.

Total flavonoids estimation. Total flavonoids were estimated according to the spectrophotometric method according to Christ and Müller [Polish Pharmacopoeia VII, 2006], expressed as quercetin equivalent (QE) per 100 g^{-1} of fresh matter (f.m.). After 45 min. the absorbance at 425 nm was measured (Hitachi U-2900 spectrophotometer (Canberra Packard). The content of flavonoids was calculated from the equation:

$$X = \frac{8.75 \cdot A}{m}$$

where: m (g) was the amount of fresh material.

Total phenolic acids estimation. Total phenolic acids estimation was carried out according to Arnov method [Polish Pharmacopoeia VI, 2002]. One milliliter of sample was mixed with 5 ml of distilled water, 1 ml 0.5 M HCl, 1 ml of Arnov reagent and 1 ml 1 M NaOH and subsequently completed to 10 ml with distilled water. The absorbance was measured at 490 nm (Hitachi U-2900 spectrophotometer (Canberra Packard). The total phenolic acid content was expressed as caffeic acid equivalent (CAE) per 100 g^{-1} of fresh matter (f.m.). The content of phenolic acids was calculated from the equation:

$$X = \frac{3.5087 \cdot A}{m}$$

where: m (g) was the amount of fresh material.

All determinations were performed in 3 replicates.

Moreover, weight of 1000 fresh broad bean seeds was determined based on the weight of 100 seeds in four replicates for each cultivar, and dry matter by means of a dryer method at 105ºC to constant weight.

Results from the two-year laboratory investigations were statistically processed as three-factorial applying the variance analysis and T-Tukey confidence intervals at 5% significance level. The paper presents results of an average for years of research.

RESULTS AND DISCUSSION

The research showed that fresh, green broad bean seeds from the pods stored for one week at 1–4°C temperature and relative humidity of 85–90%, were characterized by the same colour for all cultivars, they were firm, and in general, they showed very good quality characteristics comparable to the parameters of seeds immediately after the pod harvest and husking. Storage of the broad bean pods contributed to a change in the weight of 1000 fresh seeds. The dry matter of broad bean seeds from pods stored at 1–4°C for one week and seeds immediately after the harvest was not significantly different (tab. 1). Dry matter of broad bean seeds cultivars directly after the harvest ranged from 23.75% to 27.01% and in the seeds of stored pods ranged from 25.81% to 27.60%. The content of dry matter in seeds at the harvest was 25.99%, on average and in the seeds from stored pods was 26.53%, on average, with was optimal for the green maturity stage.

Table 1. The content of dry matter and weight of 1000 broad bean fresh seeds directly at harvest and after storage in the pods

Cultivar	Dry matter, %			Weight of 1000 fresh seeds, g		
	time of analysis					
	seeds at harvest	seeds after storage in pods	mean	seeds at harvest	seeds after storage in pods	mean
Bachus	25.45	26.75	26.10	3776	3812	3794
Basta	27.01	25.83	26.42	2607	2276	2441
R-366/1	23.75	26.25	25.00	2993	2778	2886
Bolero	26.41	25.81	26.11	3842	3486	3664
Jankiel Biały	26.52	26.96	26.74	3095	3050	3073
Windsor Biały	26.81	27.60	27.20	3766	3734	3750
Mean	25.99	26.53	26.26	3346	3189	3268
$LSD_{0.05}$						
Time of analysis (A)		n.s.		55.9		
Cultivar (B)		1.95		142.5		
A × B		3.20		233		

There were significant differences in the weight of 1000 seeds depending on the determination date and the cultivar, as well as interaction of these factors. On average, regardless of the cultivar, the seeds of broad bean from the stored pods were characterized by significantly lower weight of 1000 seeds as compared to those immediately after the harvest. The 1000 seed weight decrease of the stored pods in comparison to their shapely immediately after harvest was the highest (9.3%) for Bolero cv., while the lowest for Windsor Biały and Jankiel Biały (0.8 and 1.4%, respectively) with average for the experiment 4.7%.

Kmiecik [1994], in studies upon the refrigeration storage of broad bean pods of Windsor Biały cv. at 1–2°C temperature and relative air humidity of 85–90%, reported a gradual decrease in the 1000 seed weight, which was 4.7% after 6-day storage and was higher than results achieved in the present research.

According to [Hunter and Fletcher 2002], vegetables and other plant products are a major source of biologically active compounds that have potential antioxidant properties. Most studies of antioxidant activity in vegetables take into account antioxidants such as vitamin C and flavonoids. However, the techniques used after the harvest and before consumption, i.e. storage, freezing, or canning, determine the antioxidant activity of food. The authors showed that the antioxidant activity of extracts from pea seeds stored at 4°C was stable for 7 days after harvest. It was also shown that the antioxidant activity of aqueous extracts of vegetables such as peas, French bean, spinach, and carrots, decreased during the storage, but storage in a refrigerator at 4°C temperature resulted in a reduction in the rate of change.

It was assumed in the present study that storage of broad bean pods after harvest under low temperature of 1–4°C would keep the best quality characteristics of seeds. Results of the research showed that the content of L-ascorbic acid in the broad bean seeds from the stored pods was significantly lower and averaged to 21.50 mg 100 g^{-1} f.m. as compared to the quantity in the seeds immediately after harvesting reaching 31.27 mg 100 g^{-1} f.m. (tab. 2). However, regardless of the type of assessed seeds (immediately after the harvest and stored ones), the content of L-ascorbic acid was higher as compared to the results obtained earlier [Kmiecik et al. 1990, Łabuda 1991, Kmiecik 1994]. The high L-ascorbic acid amount in the seeds of investigated broad bean cultivars probably resulted from the favorable combination of meteorological factors during period of pods growing and filling, which was presented by Łabuda [2012]. Results achieved by Buczkowska and Sawicki [2008] on the yielding and nutritional value of sweet pepper as well as by Wierzbicka and Kuskowska [2002], in which the effect of selected factors on the content of vitamin C in different species of vegetables was evaluated, also confirm these relationships.

Table 2. The content of L-ascorbic acid and total chlorophyll in the broad bean fresh seeds directly at harvest and after storage in the pods

Cultivar	L-ascorbic acid, mg 100 g^{-1} f.m.			Total chlorophyll, mg 100 g^{-1} f.m.		
	time of analysis					
	seeds at harvest	seeds from storage in pods	mean	seeds at harvest	seeds after storage in pods	mean
Bachus	30.16	19.17	24.66	55.28	26.00	40.64
Basta	22.16	12.50	17.33	39.58	31.75	35.66
R-366/1	40.50	35.16	37.83	32.43	29.35	30.89
Bolero	39.83	25.83	32.83	48.98	25.65	37.31
Jankiel Biały	27.33	18.50	22.91	40.33	36.98	38.65
Windsor Biały	27.66	17.83	22.75	69.38	43.18	56.28
Mean	31.27	21.50	26.38	47.66	32.15	39.90
$LSD_{0.05}$						
Time of analysis (A)		0.74			0.33	
Cultivar (B)		1.89			0.86	
A × B		3.10			1.42	

Furthermore, Kmiecik et al. [1990] showed that the content of vitamin C in fresh broad bean seeds decreased along with increasing the seed ripeness, i.e. the dry matter content from 25 to 40%. In present study, dry matter content in seeds ranged from 23.75 to 27.60%, which was optimal for the green seed maturity stage [Łabuda and Łabuda 1990, Łabuda 1991].

The vitamin C content in horticultural products depends primarily on the species characteristics and cultivars, the pre-harvest factors – climatic and agronomic ones,

ripeness stage, methods of harvesting, and post-harvest factors, including storage. New methods of harvesting and post-harvest treatment should contribute to the maintenance of high nutritional quality [Lee and Kader 2000].

Total chlorophyll content in the seeds of examined broad bean cultivars immediately after harvest was significantly higher and averaged to 47.66 mg 100 g^{-1} f.m., while after pod storage, it decreased to an average level of 32.15 mg 100 g^{-1} f.m. It has been shown that seeds of Windsor Biały cv. were characterized by significantly the highest content of this component (tab. 2).

Flavonoids are secondary metabolites that are present at high levels in most plant seeds and grains [Shirley 1998]. Flavonoids and phenolic acids belong to phenolic compounds group present in vegetables, which have the greatest antioxidant properties [Chu et al. 2000 Bahourn et al. 2004, Chaieb et al. 2011]. Results achieved in the present study indicate that the broad bean seeds at technological ripeness stage were characterized by a high content of flavonoids (recalculating onto quercetin), which depending on the cultivar was 15.17–17.69 mg 100 g^{-1} f.m., on average (tab. 3). The broad bean pods storage contributed to the increase in the content of flavonoids in seeds from 15.76 to 16.31 mg 100 g^{-1} f.m., with the highest concentrations for Bolero and Jankiel Biały cultivars. The results reported by other authors [Hertog et al. 1992, Peterson and Dwyer 1998, Horbowicz 2000, Wiczkowski and Piskuła 2004] suggest that the flavonoid content in the broad bean seeds was lower ranging within 20–46 mg kg^{-1} f.m. However, the study by Chaieb et al. [2011] shows that the flavonoid content (expressed as rutin) in whole broad bean seeds depending on a cultivar ranged from 5.19 to 9.30 mg g^{-1} d.m., i.e. from 519 to 930 mg 100 g^{-1} d.m. From the study of Wang et al. [2008] great variability for flavonoids content was identified within and among legume species.

Table 3. The content of flavonoids and phenolic acids in the broad bean fresh seeds directly at harvest and after storage in the pods

Cultivar	Flavonoids, recalculated onto quercetin			Phenolic acids, recalculated onto caffeic acid		
	time of analysis (mg 100 g^{-1} f.m.)					
	seeds at harvest	seeds after storage in pods	mean	seeds at harvest	seeds after storage in pods	mean
Bachus	14.98	15.36	15.17	69.20	70.01	69.60
Basta	15.65	16.00	15.82	56.66	58.41	57.54
R-366/1	17.60	17.78	17.69	76.31	70.61	73.46
Bolero	15.66	16.73	16.20	73.63	78.56	76.10
Jankiel Biały	15.33	16.55	15.94	69.95	86.91	78.43
Windsor Biały	15.35	15.43	15.39	65.35	83.98	74.66
Mean	15.76	16.31	16.03	68.51	74.75	71.63
$LSD_{0.05}$						
Time of analysis (A)		0.10			1.60	
Cultivar (B)		0.26			4.11	
A × B		0.42			6.73	

The content of phenolic acids (recalculated onto caffeic acid) in the broad bean seeds directly after the harvest was significantly differentiated depending on the cultivars and ranged from 56.66 to 76.31 mg 100 g^{-1} f.m. (tab. 3). On the other hand, seeds from broad bean pods stored for a week were characterized by significantly higher content of phenolic acids, which ranged within 58.41–86.91 mg 100 g^{-1} f.m. Mean phenolic acid concentration (expressed as caffeic acid) in the seeds of stored broad bean pods was 74.75 mg 100 g^{-1} f.m., which was higher by 9% than the content in seeds immediately after harvest.

Similar results regarding changes to the content of free phenolic acids in fruits of eggplant cultivars after the storage were reported by Gajewski and Arasimowicz [2006]. These authors showed that the two-week eggplant fruit storage at 12°C resulted in an increase of free polyphenolic acids by about 30% as compared to the initial levels.

Lewis et al. [1999] achieved similar results of increased content of flavonoids and polyphenolic acids in potato tubers with color skin after storage at 4°C and relative humidity of 86%.

Studies performed by Chu et al. [2000] revealed that the storage of sweet potato for 7 days at 4°C resulted in a decrease of the flavonoid content (recalculated onto quercetin) from 143.78 to 91.36 mg kg^{-1} f.m.

CONCLUSIONS

Storing the broad bean pods for a week at the temperature of 1–4°C and relative humidity of 85–90% made it possible to maintain good quality characteristics of fresh green seeds. The broad bean green seeds at technological maturity stage are rich source of antioxidants the most importat of with were polyphenolic compounds, L-ascorbic acid and total chlorophyll. The content of flavonoids (QE) in the seeds at harvest ranged from 14.98 to 17.60 mg 100 g^{-1} f.m. and in the seeds of stored pods ranged from 15.36 to 17.78 mg 100 g^{-1} f.m. The content of free fenolic acid sum (CAE) in the seeds from storage pods was higher, ranged from 58.41 to 86.91 mg 100 g^{-1} f.m. and in the seeds directly at the harvest was lower, ranged from 56.66 to 76.31 mg 100 g^{-1} f.m. It has been shown that broad bean seeds from storage pods of R-366/1 cv. were characterized by significantly the higest content of L-ascorbic acid (35.16 mg 100 g^{-1} f.m.) and seeds of Windsor Biały cv. and Jankiel Biały cv. the higest content of total chlorophyll (43.18 mg 100 g^{-1} f.m. and 36.98 mg 100 g^{-1} f.m.).

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SUBSTANCJE BIOLOGICZNIE CZYNNE W ŚWIEŻYCH NASIONACH BOBU PO PRZECHOWANIU W STRĄKACH

Streszczenie. Zielone nasiona bobu w fazie dojrzałości technologicznej mają dużą wartość odżywczą – wykorzystywane są do bezpośredniego spożycia i w przemyśle przetwórczym. Jednak świeże, zielone nasiona bobu po zbiorze mają krótki okres przydatności do wykorzystania ze względu na szybkie zmiany barwy nasion. Celem pracy była ocena zawartości wybranych substancji biologicznie czynnych w świeżych nasionach bobu bezpośrednio po zbiorze oraz nasionach ze strąków przechowywanych przez tydzień w warunkach obniżonej temperatury. Materiałem doświadczalnym były świeże, zielone nasiona bobu odmian: Bachus, Basta, R-366/1, Bolero, Jankiel Biały i Windsor Biały. Nasiona bobu wysiewano w drugiej dekadzie kwietnia w rozstawie 60 × 10 cm, 100 nasion na poletku o powierzchni 6,0 m^2. Strąki bobu zbierano jednorazowo w fazie dojrzałości technologicznej nasion w drugiej, trzeciej dekadzie lipca. Z nasion handlowych pobrano próby po 500 g do badań laboratoryjnych. Równocześnie, bezpośrednio po zbiorze pobrano próby strąków handlowych bobu po 2 kg każdej odmiany do przechowywania przez 7 dni w temperaturze 1–4ºC. W nasionach bobu bezpośrednio po zbiorze oraz w nasionach ze strąków przechowywanych przez 7 dni oznaczono zawartość kwasu L-askorbinowego, chlorofilu (a + b), flawonoidów (w przeliczeniu na kwercetynę), sumy wolnych kwasów fenolowych (w przeliczeniu na kwas kawowy) i suchej masy. W wyniku przeprowadzonych badań wykazano, że świeże zielone nasiona bobu z przechowywanych strąków charakteryzowały się właściwą biało-zieloną barwą i dobrymi cechami jakości, jednakże odznaczały się istotnie mniejszą zawartością kwasu L-askorbinowego i chlorofilu (a + b). Zawartość wybranych substancji biologicznie czynnych w nasionach bobu po przechowywaniu w strąkach wykazywała istotne różnice w zależności od odmiany.

Słowa kluczowe: *Vicia faba* L. var. *major*, flawonoidy, kwasy fenolowe, chlorofil, kwas L-askorbinowy

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