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Content of amino acids in raw and frozen broad beans (*Vicia faba* var. *major*) seeds at milk maturity stage, depending on the processing method

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Abstract

The aim of the present work was to evaluate the amino acid content in products obtained from broad bean seeds, at the milk maturity stage, and the quality of the protein in broad beans. The investigation included raw seeds, fresh seeds cooked to consumption consistency and two kinds of frozen products prepared for consumption: which were obtained using the traditional method (blanching the seeds before freezing) and the ready-to-eat type (cooking the seeds before freezing). Compared with the raw material, a similar ($\alpha = 0.01$) content of amino acids in 100 g of the product was found in cooked fresh seeds. The seeds obtained from the ready-to-eat frozen product prepared for consumption contained higher amounts of all the amino acids than the traditional frozen product, and contained higher levels of some amino acids than were found in the product cooked from fresh seeds. Expressing the results per 16 g of N, the differences in the content of amino acids were much smaller. The limiting amino acid was cysteine with methionine (CS 74-86), the EAA index varying within the range 109–118.
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Keywords: Broad bean; Amino acids; Cooking; Freezing; Preparation for consumption

1. Introduction

Pulses, including broad beans, are amongst those plants regarded as being of high nutritive and biological value. They are characterized by a high content of proteins, B-group vitamins, minerals, dietary fibre and many compounds defined as non-nutrients (Chau, Cheung, & Wong, 1997; Iqbal, Khalil, Ateeq, & Khan, 2006; Prakash, Niranjan, Tewari, & Pushpangadan, 2001). Owing to the considerable variety of constituents they contain, pulses consumed in appropriate amounts can play a role in the prevention of the major diseases of affluent societies (Champ, 2001; Mathers, 2002). Most scientific publications on broad beans deal with mature seeds and the various dishes produced from them (Chau et al., 1997; Khalil & Mansour, 1995; Ziena, Youssef, & El-Mahdy, 1991). In Europe, broad beans are usually consumed before the seeds attain physiological maturity, that is, at the so-called milk maturity stage (Eurostat, 2006), corresponding to a dry matter content of 20–35% (Kmiecik, Lisiewska, & Jaworska, 1994; Lisiewska & Kmiecik, 1990). With this content of dry matter the seeds are perishable and their consumption can only be prolonged by canning or freezing (Kmiecik, Lisiewska, & Gębczyński, 1999; Lisiewska, Kmiecik, & Gębczyński, 1999).

Because of the great demand for ready-to-eat products, particularly from younger consumers, there should be a move in the food industry towards frozen products which retain the sensory traits and chemical composition of the raw material to the greatest degree, providing frozen vegetables which only require defrosting and heating rather than cooking, as was previously the case. This method of

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preparation would be quite straightforward in most households owing to the widespread use of microwave ovens (Datta, Geedipalli, & Almeida, 2005). Gębczyński (2006) showed that after 12 months' refrigerated storage, frozen broad beans obtained using the traditional method contained less vitamin C, β-carotene, polyphenols and tannins and had lower antioxidative activity than those where blanching before freezing was replaced by cooking to consumption consistency, followed by microwave defrosting and heating after the storage period. The aim of the present work was to evaluate amino acid content in products obtained from broad bean seeds at the milk maturity stage and the quality of protein in the broad beans. The investigation included raw seeds, fresh seeds cooked to consumption consistency and two kinds of frozen products prepared for consumption: obtained using the traditional method (blanching the seeds before freezing); and the ready-to-eat type (cooking the seeds before freezing).

2. Materials and methods

2.1. Materials

The materials for the experiment consisted of fresh broad bean seeds, fresh seeds cooked to consumption consistency, and frozen seeds prepared for consumption after 12 months of refrigerated storage at −20 °C. The coarse-grained cultivar Windsor Bialy was used.

The broad beans were grown in the experimental field of the research unit, where technological experiments were conducted. The field lies in southern Poland on the western outskirts of Krakow. The soil was of good horticultural structure of neutral pH and with a high content of potassium, phosphorus and calcium. In the mineral fertilization of the broad bean, the soil fertility and nutritional requirements of the crop were taken into consideration. The doses of mineral fertilizers were: 50 kg N/ha, 80 kg P_2O_5/ha and 150 kg K_2O/ha. Measures were taken during the growing period depending on the weather and tillage conditions and included sprinkler irrigation, mechanical weed control and protection against diseases and pests.

The broad beans were harvested in mid-July, when the seeds had reached a dry matter content of about 27%. As shown in earlier studies, this degree of ripeness ensures high yields and favourable sensory values for this cultivar (Kmiecik & Lisiewska, 1990). The seeds were shelled, those which were fully developed and free of blemishes being used in the experiment. The seeds were processed using two different methods.

2.2. Methods

2.2.1. Production of frozen products

Two alternative methods were used to prepare the raw material for freezing. Using the traditional technology (method I), the raw material was blanched, and, after freezing and refrigerated storage, the frozen broad beans were cooked in the traditional way. In method II, the raw material was cooked to consumption consistency before freezing, with the resulting ready-to-eat product merely requiring defrosting and heating in a microwave oven.

In method I, the broad beans were blanched in 95–98 °C water for 3 min 15 s in a stainless steel vessel, the proportion of raw material to water being 1:5. This brought about a decrease in the activity of catalase and peroxidase to a level below 5% of the initial value (Kmiecik et al., 1999). After blanching, the material was immediately cooled in cold water (14–16 °C), slightly shaken and left for 30 min on sieves to drain.

In method II, broad bean seeds were cooked to consumption consistency in 2% brine, to get the concentration of the table salt in broad beans approximately 1%. The cooking was carried out in a stainless steel vessel and the proportion of raw material to brine was 1:1. The broad beans were placed in boiling water, and the cooking time, measured from the moment when the medium came to the boil again to when the material reached the desired consistency, was 12 min. After cooking, the seeds were drained, placed in sieves and cooled in a stream of cold air (18 °C).

The materials from the blanched and cooked samples (about 10 kg/m^2) were placed on trays (30 × 50 cm) and frozen at −40 °C in a Feutron 3626-51 blast freezer, reaching −20 °C after 90 min. The frozen product was then packed in 500 g portions in polyethylene bags suitable for freezing. The bags were placed in chamber freezers and stored at −20 °C for 12 months.

2.2.2. Preparation of frozen broad beans for evaluation

Frozen blanched product was cooked in 2% brine, the proportion in weight of brine to broad bean being 1:1. As in the case of cooking, the material was placed in boiling water. The time of cooking was 8 min measured from the moment when the brine came to the boil again. After cooking, the water was immediately drained and the product was cooled to 20 °C for analysis.

A 500 g portion of the frozen broad bean product which was cooked before freezing, was defrosted and heated in a Panasonic NN-F621 microwave oven. Defrosting and heating to consumption temperature (Codex Alimentarius, 1993) took 8 min 15 s.

2.2.3. Analytical procedures

The moisture content and total N were determined according to procedures described by the AOAC (1990). The content of amino acids (except for tryptophan) was determined using an AAA-400 amino acid analyzer (INGOS, the Czech Republic). The analytical procedure applied was in accordance with the recommendations of the producer. The freeze-dried material was hydrolyzed in 6 M HCl for 24 h at 110 °C. After cooling, filtering and washing, the hydrolyte was evaporated in a vacuum evaporator at a temperature below 50 °C for sulfur-containing amino acids and below 60 °C for others amino acids, the dry residue being dissolved in a buffer of pH 2.2. The prepared sample was analyzed using the ninhydrine method. Buffers of pH 2.6, 3.0, 4.25 and 7.9 were applied. The ninhydrine solution was buffered at pH 5.5. A column 370 mm in length was filled with Ostion ANB INGOS ionex (the Czech Republic). The temperature of the column was 55–74 °C and that of the reactor was 120 °C. The determination of the sulfur-containing amino acids, methionine and cysteine, was carried out by means of oxygenating hydrolysis, using a mixture of formic acid and hydrogen peroxide (9:1) at 110 °C for 24 h. After cooling, the sample was processed as with acid hydrolysis. Buffers of pH 2.6 and 3.0 were used and the temperature of the column was 60 °C and that of the reactor was 120 °C. The calculations were carried out according to the external standard.

All determinations were carried out with four replications for each sample.

2.2.4. Expression of results

The level of each amino acid was given per 100 g of edible parts of the product, in order to compare the amino acid content in broad beans according to the culinary and technological processing applied.

The composition of amino acids was also expressed as grams per 16 g of N to estimate the quality of the protein in broad beans by comparing it with the FAO/WHO pattern (FAO/WHO, 1991; Institute of Medicine, 2002). On the basis of the amino acid composition, the CS index was calculated using the Mitchell and Block method (Osborne & Voogt, 1978), and the integrated EAA index using the Oser method (Oser, 1951).

2.3. Statistical analysis

Statistical analysis allowing a comparison of the content of amino acids in the fresh raw material, cooked raw material and frozen beans after preparation for consumption was carried out using single-factor analysis of variance (ANOVA) on the basis of the Snedecor F and Student's t tests, and the least significant difference (LSD) was calculated at the probability level $\alpha = 0.01$ and $\alpha = 0.05$ (Snedecor & Cochman, 1980). The Stastica 6.1 program was applied.

3. Results and discussion

Culinary processing, i.e. the cooking of fresh seeds, brought about minor changes in the content of amino acids, in 100 g of the product, compared with the raw material (Table 1). A significantly higher content was found in the case of arginine ($\alpha = 0.01$) and also of lysine, phenylalanine, histidine and serine ($\alpha = 0.05$), while in the case of methionine and total sulfur-containing amino acids the contents were lower ($\alpha = 0.05$). However, the total content of amino acids in cooked fresh seeds, compared with the raw material, was not significantly different. Lee, Parsons, and Downing (1982) also recorded that the level of amino acids in fresh pea seeds remained constant after thermal processing. According to Candela, Astiasaran, and Bello

Table 1
Amino acid composition of raw and processed broad beans, in mg/100 g of product

Amino acid	Seeds		Beans prepared for consumption from frozen seeds		LSD	
	Raw	Cooked	Blanched before freezing	Cooked before freezing	$\alpha = 0.01$	$\alpha = 0.05$
Isoleucine	384 ± 17	378 ± 16	300 ± 16	358 ± 11	32.4	23.1
Leucine	670 ± 15	643 ± 12	584 ± 17	697 ± 28	40.4	28.8
Lysine	564 ± 16	601 ± 21	517 ± 27	650 ± 27	50.0	35.7
Cystine	112 ± 8	104 ± 8	96 ± 5	111 ± 7	ns	10.5
Methionine	77 ± 4	68 ± 4	72 ± 3	78 ± 5	ns	6.4
Total sulphur amino acids	189	172	168	189	19.2	13.9
Tyrosine	250 ± 10	243 ± 10	203 ± 9	234 ± 7	19.9	14.2
Phenylalanine	371 ± 12	395 ± 21	336 ± 16	413 ± 9	32.7	23.3
Total aromatic amino acids	621	638	539	647	52.2	37.2
Threonine	290 ± 16	308 ± 16	269 ± 16	316 ± 20	ns	26.8
Valine	421 ± 20	410 ± 19	329 ± 17	381 ± 20	41.4	29.5
Histidine	236 ± 12	261 ± 12	231 ± 13	282 ± 11	26.0	18.5
Total essential amino acids	3375	3431	2937	3520	287.6	205.1
Arginine	1042 ± 52	1165 ± 55	962 ± 55	1193 ± 36	108.5	77.4
Aspartic acid	1044 ± 51	1066 ± 43	916 ± 47	1112 ± 27	92.3	65.8
Glutamic acid	1453 ± 74	1487 ± 77	1343 ± 66	1666 ± 46	145	103
Serine	403 ± 27	447 ± 27	403 ± 23	474 ± 20	52.6	37.5
Proline	362 ± 16	382 ± 14	332 ± 11	416 ± 18	32.0	22.8
Glycine	360 ± 19	368 ± 17	317 ± 15	378 ± 15	35.8	25.5
Alanine	386 ± 16	395 ± 15	333 ± 15	404 ± 10	30.5	21.7
Total non-essential amino acids	5050	5354	4606	5643	474.6	338.5
Total amino acids	8425	8784	7545	9163	753.7	538
Dry matter g/100 g of product	27.3	28.5	24.5	30.3		

LSD, least significant difference and ns, non-significant.

(1997), changes in the level of amino acids in fresh matter of the product due to cooking depended on the species, with only a few species being affected.

In cooked broad beans obtained from the traditional frozen product, more significant changes were recorded (Table 1). Compared with the levels found in the fresh matter of the raw material, a significant ($\alpha = 0.01$) decrease was recorded in the contents of isoleucine, leucine, tyrosine, phenylalanine, valine, aspartic acid, glycine and alanine, as well as in total sulfur-containing, aromatic, essential and total amino acids. At the $\alpha = 0.05$ level of significance, a lower contents of lysine, cystine, arginine, glutamic acid, proline and total non-essential amino acids was additionally observed. It should be noted that in broad bean seeds prepared for consumption from the traditional product, the content of dry matter was 10% lower than in the raw material and 14% lower than in cooked fresh seeds (Table 1). This was due to the "thinning" of all the constituents of dry matter including amino acids, bringing about a decrease in the content of these compounds.

Broad bean seeds prepared for consumption from the ready-to-eat frozen product contained 11% more dry matter than fresh seeds, 6% more than cooked fresh seeds, and 24% more than cooked seeds from the traditional frozen product. Thus the constituents were more "concentrated" in the product prepared for consumption in a microwave oven, as was also found by other authors (Gębczyński, 2006; Gębczyński & Lisiewska, 2006). This type of product also contained higher amounts of all the amino acids compared with cooked seeds obtained from the traditional product, the differences not being significant in the level of methionine at $\alpha = 0.01$, nor in cystine and threonine at $\alpha = 0.05$. The content of amino acids was similar to or significantly higher than that found in cooked fresh broad bean seeds or the raw material.

Much smaller changes in the content of the amino acids were found if the results were expressed not in 100 g of the products but in 16 g of N (Table 2). In broad bean seeds cooked directly after harvest, only the contents of methionine and total sulfur-containing amino acids was significantly lower at $\alpha = 0.01$, and additionally of leucine and tyrosine at $\alpha = 0.05$. Compared with raw seeds, cooked broad bean seeds from the traditional frozen product contained less isoleucine, tyrosine and valine at the $\alpha = 0.01$ level of significance, and more serine ($\alpha = 0.05$). The comparison of this product with cooked fresh seeds showed more methionine and total sulfur-containing amino acids ($\alpha = 0.01$), and more leucine at $\alpha = 0.05$. In comparison with the raw material, seeds prepared for consumption from frozen products of the ready-to-eat type – as was the case with cooked seeds obtained from the traditional frozen product – contained less isoleucine, tyrosine, valine ($\alpha = 0.01$) and, at $\alpha = 0.05$, less leucine and both the sulfur-containing amino acids. The levels of amino acids in this sample showed changes of a similar order when compared with the levels in both cooked fresh seeds and the raw material, only the levels of leucine and sulfur-containing amino acids remaining constant.

A comparison of the amino acid content in cooked seeds, obtained from frozen product showed that the ready-to-eat product contained less methionine ($\alpha = 0.01$), leucine, total sulfur-containing amino acids and tyrosine ($\alpha = 0.05$) than the traditional frozen product. During the entire technological and culinary process, the levels of lysine, phenylalanine, all the non-essential amino acids and also total amino acids remained constant.

According to Chau et al. (1997), Mutia and Uchida (1993) and Porres et al. (2002), thermal processing brought about an increase, decrease or variation in the content of different amino acids expressed in 16 g of N depending on the time of heat treatment and the species of the pulse. Wu et al. (1996) postulated that – as in the present paper – the cooking of red kidney beans did not significantly change the content of most amino acids. Moreover, as in the present investigation, the levels of lysine and proline remained constant irrespective of the parameters of the heat treatment. Different results were reported by Khalil and Mansour (1995), who found that the cooking of dry broad bean seeds brought about an increase in the contents of leucine, threonine and histidine. The present authors (Kmiecik et al., 1999, 1994) found in earlier studies that the changes brought about by thermal processing depended on the cultivar and maturity of the broad bean seeds.

The nature and extent of changes in the amino acid content of products prepared for consumption compared with those in the raw material did result from heat treatment, but their level might also have been affected by the solubility of protein in the water, or possibly by damage to the seed coat (Ruiter, Schuurmans, & Kollöffel, 1984). It has also been claimed that the decomposition of some protein fractions rich in essential amino acids results in higher values for non-essential amino acids when they are calculated on the basis of 16 g of N (Mutia & Uchida, 1993).

Comparing the content of amino acids with the standard (FAO/WHO, 1991; Prakash et al., 2001) can also be used as a method to evaluate the nutritional value of protein. Investigators did not always use the same standard, therefore a comparison of their results with the present data is not always possible. However, there is no doubt that in pulses generally, the limiting amino acids of the 1st order are the sulfur-containing ones, while the limiting amino acid of the 2nd order in the case of broad beans is threonine (Kmiecik et al., 1994; Lisiewska et al., 1999).

The protein in broad bean seeds, both raw and prepared for consumption, was of high quality. Cysteine with methionine was the limiting amino acid of the 1st order (Table 3). However, the value of the CS index for these amino acids was high and varied from 74 for fresh cooked seeds to 86 for the raw material and cooked seeds obtained from the traditional frozen product. Threonine was the limiting amino acid of the 2nd order, the CS value for this amino acid ranging from 95 for the cooked ready-to-eat product to 101 for the cooked traditional product. However, the

Table 2
Amino acid composition of raw and processed broad beans, in g/16 g of N

Amino acid	Seeds		Beans prepared for consumption from frozen seeds		LSD	
	Raw	Cooked	Blanched before freezing	Cooked before freezing	$\alpha = 0.01$	$\alpha = 0.05$
Isoleucine	4.35 ± 0.19	4.09 ± 0.18	3.84 ± 0.20	3.67 ± 0.11	0.372	0.265
Leucine	7.60 ± 0.17	7.16 ± 0.12	7.48 ± 0.21	7.15 ± 0.28	ns	0.320
Lysine	6.41 ± 0.17	6.49 ± 0.23	6.62 ± 0.35	6.67 ± 0.27	ns	ns
Cystine	1.28 ± 0.09	1.12 ± 0.08	1.23 ± 0.06	1.14 ± 0.07	ns	0.114
Methionine	0.87 ± 0.04	0.73 ± 0.04	0.92 ± 0.04	0.80 ± 0.05	0.098	0.070
Total sulphur amino acids	2.15	1.85	2.15	1.94	0.221	0.157
Tyrosine	2.84 ± 0.12	2.63 ± 0.11	2.60 ± 0.12	2.40 ± 0.07	0.229	0.163
Phenylalanine	4.20 ± 0.14	4.26 ± 0.23	4.30 ± 0.20	4.23 ± 0.09	ns	ns
Total aromatic amino acids	7.04	6.89	6.90	6.63	ns	ns
Threonine	3.29 ± 0.18	3.34 ± 0.18	3.44 ± 0.20	3.24 ± 0.21	ns	ns
Valine	4.79 ± 0.23	4.43 ± 0.21	4.22 ± 0.22	3.91 ± 0.20	0.467	0.333
Histidine	2.68 ± 0.13	2.82 ± 0.13	2.96 ± 0.17	2.89 ± 0.12	ns	ns
Total essential amino acids	38.31	37.07	37.61	36.10	ns	ns
Arginine	11.8 ± 0.59	12.6 ± 0.59	12.3 ± 0.71	12.2 ± 0.37	ns	ns
Aspartic acid	11.85 ± 0.58	11.5 ± 0.46	11.7 ± 0.60	11.4 ± 0.27	ns	ns
Glutamic acid	16.5 ± 0.84	16.55 ± 0.83	17.2 ± 0.85	17.1 ± 0.47	ns	ns
Serine	4.57 ± 0.30	4.83 ± 0.29	5.16 ± 0.29	4.86 ± 0.22	ns	0.419
Proline	4.11 ± 0.18	4.13 ± 0.15	4.25 ± 0.14	4.27 ± 0.18	ns	ns
Glycine	4.09 ± 0.22	3.98 ± 0.18	4.06 ± 0.19	3.87 ± 0.15	ns	ns
Alanine	4.38 ± 0.18	4.28 ± 0.16	4.27 ± 0.20	4.14 ± 0.10	ns	ns
Total non-essential amino acids	57.31	57.89	58.98	57.86	ns	ns
Total amino acids	95.62	94.97	96.59	93.96	ns	ns
N g/100 g of dry matter	5.16	5.18	5.08	5.15		

LSD, least significant difference and ns, non-significant.

Table 3
Amino acids indexes of raw and processed broad beans according to FAO/WHO (1991)

Index	Amino acid	Seeds		Beans prepared for consumption from frozen seeds	
		Raw	Cooked	Blanched before freezing	Cooked before freezing
CS	Izsoleucine	155	146	137	131
	Leucine	115	108	113	108
	Lysine	111	112	114	115
	Cystine + methionine	86	74	86	78
	Tyrosine + phenylalanine	112	109	110	105
	Threonine	97	98	101	95
	Valine	137	127	121	112
	Histidine	141	148	156	152
EAA		118	113	116	109

CS, chemical score index and EAA, essential amino acid index.

EAA value was only 6% lower for the cooked ready-to-eat product and only 3% lower for cooked fresh seeds.

4. Conclusions

Compared with the raw material, a similar ($\alpha = 0.01$) content of amino acids, in 100 g of product, was found in cooked fresh seeds. The seeds obtained from the ready-to-eat frozen product prepared for consumption contained higher amounts of all the amino acids compared with the traditionally frozen broad beans, and of some amino acids compared with cooked fresh seeds. Expressing the results per 16 g of N, the differences in the content of amino acids were much smaller. The limiting amino acid was cysteine with methionine (CS 74-86), the EAA index varying within the range 109–118.

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