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Study on Amino Acid Content in Selected Varieties of *Pisum sativum* (peas) by Ion Exchange Chromatography

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Abstract. The *Pisum sativum* (Peas), is a widely grown legume in the Central Europe. Many countries spend high expenditure for importing *Glycine max* (Soya beans) because it is the most popular and main protein source for vegetarians as well as animal feed. The search for an alternative source of plant protein is important to cut down the expenditure, reduce protein energy malnutrition and to maintain the animal production as well. Amino acid analysis is a good indicator to measure the quality of protein which may vary according to different species and environmental factors. Therefore, the objectives of the present study is to characterization of *Pisum sativum* varieties grown in Central Europe by evaluating amino acids as an indicator of protein quality and to compare them with *Glycine max* (soya) and FAO reference pattern to find the possibility of using it as an alternative plant protein source. Seeds of four varieties *Pisum sativum* (Terno, Xantos, Svit, Achat) were analysed for their amino acid profiles in dried seeds. All legume samples were ground by using a laboratory grinder and they were subjected to acid hydrolysis with 6 M HCl at 110°C for 23 hours. Amino acids containing sulphur were determined separately in 6 M HCl after oxidative hydrolysis (formic acid + hydrogen peroxide, 9:1 v/v, 20 h at 4°C). Amino acids were determined using an AAA 400 amino acid analyser (INGOS, Czech Republic) with ion exchange chromatography with post column ninhydrin-based detection. The ninhydrin amino acid derivatives are purple for primary amino acids (detection at 570 nm) and yellow for secondary amino acids (detection at 440 nm). The results of *Pisum sativum* (Terno, Xantos, Svit, Achat) were compared with *Glycine max* that grown in Central Europe, *Pisum sativum* (green peas) grown in Asia and FAO/WHO reference pattern. The most of all essential amino acid profile of total seed proteins compared favourably with FAO/WHO requirements except phenylalanine in all the varieties. The highest total essential amino acids (47.9g/16gN) is in *Pisum sativum* (Terno) among all the *Pisum sativum*, *Glycine max* tested (45.6g/16gN) and FAO/WHO reference pattern. Methionine content is higher in *Pisum. sativum* (Terno) i. e. 5g/16gN among all the *Pisum sativum* and FAO/WHO reference pattern. Total sulphur containing amino acids of Terno is higher than FAO/WHO reference pattern. *Pisum sativum grown* in Central Europe are rich in lycine, leucine and arginine and can fulfill the essential amino acid content of human diet.

Keywords: *Pisum sativum*, Amino acid, Acid hydrolysis, Oxidative hydrolysis, Ion exchange chromatography

1. Introduction

In developed countries, plant proteins are now regarded as either versatile functional ingredients or as biologically active components, rather than as essential nutrients. This evolution towards health and functionality, which is mainly driven by the partial replacement of animal foods with legumes, has shown to improve nutritional status (Guillon and Champ, 2002) due to low cholesterol level in plant foods, and increased level of fibre intake which reduces the risk of bowel diseases, including cancer (Adebowela *et al*., 2007).

The imported *Glycine max* (soybean) is the main protein source for vegetarians as well as animal feed in many countries and therefore, need to spend high expenditure on feed for animal production. Further, the

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animal production which is the main source of protein for non-vegetarians has declined due to insufficient animal feed. Hence, the search for alternative sources of protein is important to maintain the animal production as well. Among the legumes, *Pisum sativum* (pea) is the most widely grown legume in Central Europe though the consumption is low when compared to other sources of proteins. Some researchers have reported that *Pisum sativum* is an excellent source of nutrients for all species of animal with high digestibility and palatability (Chrenkova *et al*., 2007).

Though many studies have been carried out in other parts of the world, there is limited information on characterization of *Pisum sativum* varieties grown in Central Europe by evaluating amino acid profiles.

Therefore, the objectives of the present study is to characterization of *Pisum sativum* varieties grown in Central Europe by evaluating amino acids as an indicator of protein quality and to compare them with *Glycine max* (soya) and FAO reference pattern to find the possibility of using it as an alternative plant protein source.

2. Methodology

Legume samples of *Pisum sativum* (Terno, Xantos, Svit, Achat) and *Glycine max* used for this study were ground and preserved in air-tight bottles at room temperature.

All ground legumes samples were subjected to acid hydrolysis with 6 M HCl at 110°C for 23 hours. Amino acids containing sulphur was hydrolysed separately with 6 M HCl after oxidizing (formic acid + hydrogen peroxide, 9:1 v/v, 20 h at 4±1°C). Amino acids were determined by using an AAA 400 amino acid analyser (INGOS, Czech Republic) with ion exchange chromatography with post column ninhydrin-based detection by using Sodium citrate buffer. The ninhydrin amino acid derivatives were detected at 570 nm for primary amino acids and at 440 nm for secondary amino acids (Bunka *et.al*, 2004)

3. Results and Discussion

Table 1 shows the total amino acid composition of some varieties of *Pisum sativum* seeds and that of the FAO/WHO 1991 reference pattern. All *Pisum sativum* seeds used in this study had relatively high essential and non-essential amino acids, with the exception of cysteine and methionine. The observation of relatively low concentrations of methionine and cysteine in legumes has been reported many researchers (Apata and Ologhobo, 1994) The data presented here also show that, sulphur amino acids, which were in the shortest supply and first limiting. However, the methionine content of variety Terno (5.00 ± 0.061g/16gN) was higher than that of other varieties of *Pisum sativum* tested, FAO/WHO 1991 reference pattern and in green peas tested by Iqbal et al 2006.

Most of all essential amino acids were higher than the FAO/WHO 1991 reference pattern except phenylalanine. Phenylalanine was particularly low in all varieties than FAO/WHO 1991 reference pattern while arginine was much higher.

When compared with earlier studies on *Pisum sativum* by Iqbal *et al*. 2006 in Pakistan, there was somewhat lower content of essential and none essential amino acids in three varieties tested (Xantos, svit, Ahat) while arginin was higher This may be due to soil and climatic difference of Asian and Europe or varietal differences. Many investigators have reported that environmental factors under which food legumes grown could influence their amino acid composition (Oshodi, *et al*.1995; Chau *et al*. 1998). Arginine content was ranged from 8.32-9.68/16gN in all varieties of peas tested and it is above the FAO/WHO 1991 reference pattern (1.1/16gN) and *Glycine max* tested (7.74/16gN) that grown in Central Europe.

Lysine levels ranged from 6.39- 6.93/16gN in all the varieties of *Pisum sativum* (peas) tested. Though it is slightly lower than the past study of Iqbal *et al*.2006, it is above the FAO/WHO 1991 reference pattern and *Glycine max* tested that grown in Central Europe. The high content of lysine noted in these legumes supports the results of other investigators, and the amount obtained for this amino acid is more or less similar to the content of lysine in soya i.e. 6.08g/16gN (Siddhuraju and Becker, 2005). The lowest value, lysine reported for the variety Svit, exceeded the reference amino acid pattern and soya bean. The importance of low-cost sources of plant protein high in lysine is quite important as low content of lysine in cereals which is the staple diet in many parts of the world. Therefore, cheap sources of lysine such as the legume seeds studied

are of primary importance to us for the successful feeding of our expanding population. This should be especially true if all of the lysine is available.

Table 1. Comparison of amino acid composition of some varieties of *Pisum sativum* grown in Central Europe with grean peas (Asia), *Glycine max* and FAO reference pattern (g/16g of N)

Amino acid	*Pisum sativum*				Green Pea Iqbal et al.2006	*Glysine max* in Central Europe	FAO/ WHO 1991 Refer @
	Terno	Xantos	Svit	Achat			
(EAA)							
Arginine	9.36 ± 0.22	8.60 ± 0.45	9.68 ± 0.22	8.32 ± 0.45	7.2± 0.04	7.74± 0.33	1.1
Histidine	2.22 ± 0.12	2.16± 0.11	2.18 ± 0.01	2.16 ± 0.08	2.4±0.05	2.36 ± 0.13	1.9
Isoleucine	4.23 ± 0.09	3.86 ±0.19	3.77 ±0.02	3.90± 0.09	4.5±0.06	4.24 ± 0.17	2.8
Leucine	7.11 ± 0.16	6.45 ±0.29	6.33 ± 0.07	6.55 ± 0.21	7.4±0.05	6.94± 0.25	6.6
Lysine	6.93 ± 0.20	6.55 ±0.26	6.39 ± 0.03	6.63 ± 0.21	8.1±0.07	5.79 ± 0.30	5.8
Methionine	5.00 ± 0.06	1.08 ± 0.01	1.05±0.06	0.99 ± 0.05	1.1±0.03	5.54±0.10	2.5+Cyst
Phenylalanine	4.87± 0.27	4.59 ± 0.20	4.33 ± 0.04	4.56± 0.13	5.2±0.04	4.69 ± 0.19	6.3
Threonine	3.45±0.029	3.64±0.16	3.34±0.14	3.53±0.12	3.8±0.05	3.62±0.20	3.4
Tryptophan	n.a	n.a	n.a	n.a	0.8±0.02		1.1
Valine	4.72 ± 0.07	4.29 ± 0.19	4.34 ± 0.05	4.32 ± 0.17	5.0±0.09	4.70 ± 0.23	3.5
Total (EAA)	47.89	41.22	41.41	40.96	45.5	45.62	35
NEAA							
Alanine	4.19± 0.16	3.88 ± 0.17	3.83 ± 0.04	3.79 ± 0.24	5.2±0.04	3.99±0.20	
Aspartic acid	10.87±0.13	10.55±0.34	10.69± 0.09	10.58±0.43	11.0±0.06	10.29±0.41	
Cysteine	2.01±0.02	2.03± 0.06	1.90 ± 0.08	1.67 ± 0.14	1.8±0.03	2.40± 0.03	
Glutamic cid	15.07±0.26	16.19±0.67	15.96 ±0.85	16.16±0.68	17.5±0.06	15.61±0.73	
Glycine	4.11± 0.05	4.00± 0.14	3.98± 0.03	3.92± 0.21	4.5±0.01	4.33± 0.17	
Proline	3.77 ± 0.12	3.57± 0.15	3.64 ±0.12	3.63± 0.14	3.8±0.03	4.60± 0.41	
Serine	4.23 ±0.06	4.16 ± 0.18	4.05± 0.06	4.25± 0.22	5.1±0.54	4.33±0.17	
Tyrosine	2.79 ± 0.10	3.18± 0.23	2.87 ± 0.11	3.18± 0.24	3.7±0.03	2.85±0.11	
Total (NEAA)	47.04	47.56	46.87	47.18	52.9	39.47	

EAA – Essential Amino Acids
NEAA – Non Essential Amino Acids

@ Data from FAO/WHO (1991) reference pattern of essential amino acid requirement for pre-school children (2–5 years old).
+Cyst =figure 2.5 has noted for total of methionine and cysteine amino acid
Data in each column contains mean and standard deviation of mean

The highest content of Leucine (7.11/16gN) was noted in variety Terno among all the varieties of peas tested and it is above the FAO/WHO 1991 reference pattern and *Glycine max* tested that grown in Central Europe.

Though it has reported that *Glycine max* (soya) is as one of the most important legumes in nutritional standpoints (Frias *et al*. 2008) all the EAA are relatively higher in all varieties of peas tested when compared the EAA in *Glycine max* tested that grown in Central Europe. Variety Terno had higher EAA than EAA in

Glycine max while other varieties had somewhat lower EAA than *Glycine max.* However, NEAA is higher in all varieties tested when compared with *Glycine max.*

The above analytical data revealed, that *Pisum sativum* that grown in Central Europe are rich in lysine, leucine and arginine

4. Conclusion

The most of all essential amino acid profile of total seed proteins compared favourably with FAO/WHO requirements except phenylalanine in all the varieties. The highest total essential amino acid content (47.9g/16gN) is in *Pisum sativum* (Terno) among all varieties of *Pisum sativum* which is higher than *Glycine max* (soya bean) tested and FAO/WHO reference pattern.

Methionin content is highest in *Pisum sativum* (Terno) i. e. 5g/16gN among the *Pisum sativum* varieties including Pakistan variety. Sulphur containing amino acids are higher in the variety Terno when compared with other *Pisum sativum* and FAO/WHO reference pattern. Lysine levels ranged from 6.39- 6.93/16gN in all varieties of peas tested which is higher than *Glycine max* (soya bean) tested and FAO/WHO reference pattern.

Pisum sativum grown in Central Europe are rich in lycine, leucine and arginine and can fulfill the essential amino acid content of human diet.

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