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Study of Amino Acid Profile and Solubility of Pea Protein Isolate for the Production of Beverages for Psychiatric Patients

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Abstract. Excessive intake of saturated fatty acids and physiologically imbalanced amino acids worsens the patient's psychotic condition, creating the risk of developing psychosis and other comorbidities. Therefore, to ensure the optimal amount of amino acids and improve the psychotic state, pea protein isolate is an alternative to nutritional supplementation. The aim of the study was to analyse the amino acid profile of pea protein isolates and their solubility to evaluate their potential for the development of beverages for patients with psychiatric disorders. In the study, the amino acid profile analysis of organic and conventional pea protein isolates was performed using high performance liquid chromatography mass spectrometry. To evaluate solubility, pea protein isolates were treated in an ultrasonic bath and at different pH concentrations. The results showed that organic pea protein isolate showed a higher composition of essential amino acids (41.27%) and semi-essential amino acids (8.37%) than conventional pea protein isolate. Organic pea protein isolate was able to provide a more appropriate amino acid composition to meet the amino acid needs of patients with psychiatric disorders. Organic and conventional pea protein isolates had higher solubility in the ultrasonic bath of 20 min and in the alkaline environment. The solubility between the two pea protein isolates was not significantly different, but the ultrasonic bath treatment and different pH concentrations were significant, which gave insight and further application in beverage production.
Key words: pea protein isolate, psychiatry, amino acid, pH, solubility, functionality.

Introduction

Pea protein isolate is a good dietary supplement alternative because it contains all essential amino acids, and semi-essential amino acids, is highly nutritious, not genetically modified (Lam et al., 2018), lactose-free, gluten-free and suitable for vegans and vegetarians (Stilling, 2020). Amino acids play a vital role in psychiatric health because they participate in the synthesis of hormones and neurotransmitters that regulate behavioural processes (Nimgampalle et al., 2023). Neurotransmitters are involved in a wide range of physiological processes, including the regulation of cognitive functions, sensory perception, and mood regulation (Nimgampalle et al., 2023). Optimal amino acid intake from a complete diet is essential to improve overall health, cognitive function, and prevent various health conditions associated with physiologically adequate amino acid intake.

Patients with psychiatric disorders have unbalanced nutritional principles, which, in the long term, can lead to comorbidities such as obesity and malnutrition (Mötteli et al., 2023). Studies show that increased consumption of red meat increases anxiety and worsens psychotic conditions (Darooghegi-Mofrad et al., 2021). Although animal proteins have a high nutritional value, and are a source of zinc and iron, their excessive use provides a high amount of saturated fatty acids, which can increase the development of chronic diseases (Xiao et al., 2023). Eating high amounts of red meat can create an imbalance in arachidonic acid, which increases inflammatory processes and the risk of depression (Shibata et al., 2018). Studies have shown that patients with moderate depression have reduced blood concentrations of essential amino acids such as histidine, leucine, and isoleucine (Solís-Ortiz et al., 2021). Amino acids such as glycine, serine, glutamate, and cysteine play an important role in the metabolism of homocysteine, and the formation of glutathione, which serves as an antioxidant against cell apoptosis (Godwin & Geisinger, 2017).

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The effects of methionine on microvascular dysfunction, synaptic function, and neuronal health are increasingly being studied (Ungvari et al., 2023). Research suggests that methionine restriction and supplementation show promising results, and its regulation is essential for maintaining a healthy life (Navik et al., 2021). Long-term low methionine intake leads to bone fragility, while increased methionine intake leads to hyperhomocysteinemia and elevated cholesterol levels (Navik et al., 2021). The methionine content of pea protein isolate was lower than recommended by the WHO (World Health Organization) (Gorissen et al., 2018). Therefore, to ensure physiologically adequate methionine intake without contributing to cerebrovascular disease and neurotransmitter deficiency (Yu et al., 2022), adequate dietary intake of plant, including pea protein isolate, and animal products is required to balance methionine levels (Yu et al., 2022). Leucine stimulates muscle protein synthesis and can help prevent muscle wasting (Sunsandee et al., 2024). Studies have shown that dietary supplementation with the essential amino acids L-lysine (one of the two optical isomers of lysine) and L-arginine (one of the two optical isomers of arginine) reduced stress-induced anxiety (Smriga et al., 2007). Phenylalanine is involved in the formation of neurotransmitters, promotes the proper functioning of the nervous system, improves motivation, increases concentration, reduces anxiety and improves mood (Akram et al., 2020). A clinical study of 18 individuals divided into two groups found that the group who consumed plant-based meals during the test had higher levels of brain tryptophan and tyrosine than those who consumed meals high in animal foods (Sheikhi et al., 2023). The amino acid arginine, which is present in pea protein isolate, is a semi-essential amino acid, because its endogenous synthesis is sufficient under normal physiological conditions, but during embryogenesis and catabolic stress, the body is unable to synthesise the necessary concentrations, so arginine must be supplemented with food (Morris, 2007).

Considering the above, it is essential to evaluate the appropriate provision of amino acids for patients with psychiatric disorders. The pea protein can be considered a high quality protein because it has a balanced ratio of amino acids and all essential amino acids that meet the recommendations of FAO (Food and Agriculture Organization) and WHO (World Health Organization) (Gorissen et al., 2018). The rate of digestion of pea protein isolate has been studied in comparison to several other proteins. Pea protein is an excellent source of protein with high bioavailability, excellent digestibility and, unlike animal proteins, offers beneficial long-term effects on human health and the global ecology. As one of the factors that can affect the availability of pea protein isolate, there are antinutritional factors that inhibit the absorption and digestibility of nutrients (Salles et al., 2021). According to studies, pea protein isolate contains some antinutritional factors that do not affect digestibility, but their presence and effect should be considered (Gultekin Subasi et al., 2024). The in vitro digestibility of pea protein was 97.3%, which was very similar to the digestibility of casein protein of 98.0%. In general, the digestibility of pea protein is very similar to egg and soy protein and higher than other vegetable products (Guillin et al., 2022).

Solubility is one of the most widely evaluated functional qualities of food proteins as it affects their functional properties such as foaming, emulsification, and gelation (Kuang, 2022). The solubility of pea protein is highly dependent on pH, because depending on it, fruit, vegetable and berry powders and juices of the appropriate pH concentration can be selected and adjusted. Studies generally demonstrate higher protein solubility of pea protein isolate from pH 9.0 to pH 11.0, but poor solubility at pH 2.0, which will provide a basis for evaluating pH concentration in beverage production (Sajib et al., 2023).

The aim of the study was to analyse the amino acid profile of pea protein isolate and its solubility to evaluate its potential for development of beverages for patients with psychiatric disorders.

Materials and Methods

Materials

Pea protein isolate was purchased according to the following criteria - unflavoured, without specific taste, protein amount greater than 74 g 100^{-1} product, powdery consistency and with no added sweeteners, vitamins or minerals. Two pea protein isolates were purchased in an online store, initially coordinating with the distributor to ensure the criteria were met.

Organic pea protein from the Netherlands, obtained by extracting proteins from yellow peas, hypoallergenic and the peas from which they are produced are not genetically modified. Conventional pea protein isolate is produced in China, obtained by extracting proteins from Canadian yellow field peas. The nutrition facts of the two mentioned pea protein isolates are summarised in table for a clearer overview (Table 1).

Determination of amino acid profile in pea protein isolate

Amino acid composition was determined using high-performance liquid chromatography - mass spectrometry (HPLC-MS) according to the methodology described by Ozolina (Ozolina et al., 2024).

Evaluation of the solubility of the pea protein isolates at different times in the ultrasonic bath:

In a 50 ml beaker, 1g of pea isolate was mixed with 10 ml of distilled water. The isolate was tested

Table 1

Nutrition facts of organic and conventional pea protein isolate

Nutrition facts	Organic pea protein g 100 g^{-1} product	Conventional pea protein isolate g 100 g^{-1} product
Energy value	374 kcal	399 kcal
Fat	0.5	1.9
Carbohydrates	14.0	2.8
Sugar	0.7	0.0
Fibre	3.0	1.0
Proteins	81.3	85.1
Salt	0.5	n.d.
Dry matter content	92.79 %	92.48 %
Crude protein content	81.88 %	82.49 %.

n.d - not defined

in the 6L ultrasonic bath set (WUC – D06H, Korea) at three different times, with three replicates. A control sample was prepared where the sample was not subjected to the ultrasonic bath treatment. Three samples were prepared, each with time intervals of 5 min, 10 min, and 20 min, which were treated in an ultrasonic bath at 425 power (W), 50 kHz and 90% amplitude. After the ultrasonic bath, the samples and the control sample were weighed into tubes and centrifuged (ELMI CM-6MT, North America) at 2300 × *g* for 20 min. After centrifugation, the liquid content of each test tube was weighed on Petri plates and left to dry in a drying oven (ETUVE MEMMERT UF55, Germany) for 24 hrs at 105°C, fan power 100%. After drying, each sample was weighed, and the dried mass was calculated. The methodology on which the solubility assessment was based was taken from (Gao et al., 2022; Yang et al., 2022). Solubility was calculated according to the following equation, which shows the proportion of protein dissolved in a given solvent under specific conditions:

$$\text{Protein solubility (\%)} = (P_1 / P_2) \times 100$$

where:
P_1 – protein content in the supernatant, %;
P_2 – total protein, %.

Solubility of pea protein isolate in water

The solubility of the pea protein isolate was tested at different pH concentrations (pH 3.0, pH 5.0, pH 7.0, pH 9.0, pH 11.0), with three repetitions from each sample. In a 50 ml beaker, 0.25 g of pea protein isolate was mixed separately with 10 ml of distilled water and the pH was adjusted to 3.0 - 11.0 using 1M NaOH or 1M HCl. Protein dispersions were mixed using a magnetic stirrer for 20 min at a temperature of 22 °C (measurement was made with an infrared temperature meter). The dispersions were placed in a centrifuge at 2300 × *g* for 20 min. After centrifugation, the liquid content of each test tube was weighed on Petri plates and left to dry for 24h (at 105 °C, fan power 100%). After drying, each sample was weighed and the dried mass was calculated, the average values from each pH concentration were calculated. The methodology on which the solubility assessment was based was taken from (Gao et al., 2022; Sajib et al., 2023; Yang et al., 2022).

Statistical analysis

Arithmetic mean and standard deviation were calculated for the results obtained in the study. Analysis of variance (ANOVA) using a *P* value of 0.05 was applied to evaluate the significant differences between the amino acid compositions of the samples. Tukey's test was used to determine significant differences (*P value* at 0.05) between the duration (time) of treatment in the ultrasonic bath and the control group in each sample.

Results and Discussion

A significant difference in the amino acid profile was observed between organic and conventional pea protein isolate ($p<0.05$). Organic pea protein isolate had a higher content of essential amino acids than conventional pea protein isolate, except for threonine and histidine (Table 2).

The leucine content in organic pea protein isolate was 0.6% higher than that of conventional pea protein isolate. Compared to other plant products, the leucine content of pea protein isolate is higher than that of soybean (5.0%), oat (3.8%) and hemp protein (2.6%) (Asen et al., 2023). In terms of health effects, taking 4.5 g of leucine per day for 12 weeks increased

Table 2

Amino acid profile of organic and conventional pea protein isolates and comparison with literature data and recommendations

Amino acids	Organic pea protein isolate		Conventional pea protein isolate		Pea protein isolate, g 100g^{-1} DM ***	Recommended daily dose for adults mg/kg/day	Required amino acid content (g) for an adult weighing 70 kg****
	g 100 g^{-1} DM	%	g 100 g^{-1} DM	%			
Essential amino acids							
Valine	4.15±0.04^{a}	5.65	3.94±0.05^{b}	5.23	2.7 – 5.0	10	0.70
Leucine	6.78±0.06^{a}	9.22	6.48±0.01b	8.62	5.7 - 6.4	14	0.98
Isoleucine	3.95±0.00^{a}	5.37	3.75±0.04^{b}	4.99	2.3 - 4.5	10	0.70
Methionine	0.80±0.01^{a}	1.09	0.70±0.05^{b}	0.93	0.3 - 1.1	13	0.91
Phenylalanine	4.49±0.06^{a}	6.11	4.28±0.05^{b}	5.69	3.7 - 5.5	14	0.98
Threonine	2.62±0.03^{b}	3.57	2.71±0.06^{a}	3.60	0.7 – 1.0	7	0.49
Lysine	5.88±0.10^{a}	8.00	5.67±0.02^{b}	7.54	4.7 - 5.7	12	0.84
Histidine	1.66±0.05^{b}	2.26	1.77±0.07^{a}	2.35	1.6 - 2.5	8 - 12	0.56 - 0.84
Total	30.33^{a}	41.27	29.30^{b}	38.92	-	-	-
Semi-essential amino acid							
Arginine	6.15±0.13^{a}	8.37	6.27±0.04^{b}	8.33	6.6	Safe daily dose ~ 20g/day**	n.d.
Unessential amino acids							
Cystine	0.71±0.03^{b}	0.96	0.81±0.07^{a}	1.08	0.2 - 1	4.1 *	n.d.
Serine	3.73±0.22^{b}	5.07	4.00±0.08^{a}	5.32	3.6 - 5.3	n.d.	n.d.
Aspartic acid	8.18±0.15^{b}	11.12	8.98±0.22^{a}	11.94	8.9 - 11.5	n.d.	n.d.
Glycine	2.89±0.12^{a}	3.93	2.61±0.07^{b}	3.47	2.8 - 4.1	n.d.	n.d.
Glutamic acid	12.75±0.14^{b}	17.34	14.10±0.13^{a}	18.75	12.9 - 13.2	n.d.	n.d.
Alanine	3.16±0.06^{b}	4.30	3.33±0.04^{a}	4.43	3.2 - 4.3	n.d.	n.d.
Proline	2.89±0.09^{b}	3.94	3.23±0.01^{a}	4.29	3.1 - 4.5	n.d.	n.d.
Tyrosine	2.71±003^{a}	3.69	2.57±0.06^{b}	3.42	2.6 - 3.8	n.d.	n.d.
Total	37.02^{b}	50.35	39.63^{a}	52.7	-	-	-

Significant differences were sought between columns 2 and 4 ($p<0.05$) and are indicated by the letters a, b in each cell.
** (EFSA Panel on Dietetic Products, 2012).*
*** (McNeal et al., 2016).*
**** (Babault et al., 2015).*
***** For an adult (the highest, average value for both women and men) with an activity coefficient of 1.4 (Ministry of Health of Latvia), without malnutrition.*
n.d - not defined.

muscle mass in patients with observable sarcopenia (Sunsandee et al., 2024). Therefore, pea protein has great potential, because the amount of lysine in it could provide the necessary daily norm for an adult - 12 mg/kg/day. In a study comparing whey protein with pea protein, it was observed that pea protein also increased biceps brachii, which is a good alternative and applicable to patients with nutritional deficiencies caused by disease (Babault et al., 2015).

The next amino acid with a high concentration was lysine, 8.00% organic, which is 0.46% more than in conventional pea protein isolate. In other studies, the concentration of lysine in yellow peas was 4.7%, and in soybeans it was 3.2% to 3.4%, which is lower compared to organic pea protein isolate (Asen et al., 2023). Studies have shown that taking 6 g of L-lysine per day as a supplement for one week reduced hallucinations, nightmare episodes and improved thinking in patients with schizophrenia (Sunsandee et al., 2024). Supplementing the diet with the essential amino acids L-lysine - 2.64 g and L-arginine - 2.64 g per day reduced the level of anxiety and improved the wellbeing of the patients (Smriga et al., 2007). The amount of phenylalanine in the organic pea isolate was 6.11%, which was 0.42% more than in the conventional pea isolate. The amount of phenylalanine in other studies was 2.41% lower than in organic pea protein isolate (Gorissen et al., 2018). Phenylalanine is involved in the formation of neurotransmitters, promotes the proper functioning of the nervous system, improves motivation, increases concentration, reduces anxiety, and improves mood (Akram et al., 2020). The valine content of organic pea protein isolate was 5.56% and the isoleucine content was 5.37%, compared to conventional pea protein isolate, which had 0.33% less valine and 0.38% less isoleucine. The content of methionine in organic pea protein isolate - 1.09% and conventional pea protein - 0.93% was approximately 1.2% lower than the daily recommended dose (Word Health Organization, 2002) (Table 2). The daily requirement for methionine in adults is approximately 13 mg/kg/day, although approximately 30% of the adult human requirement for methionine can be replaced by cysteine (Young & Shalchi, 2005). Between the two pea protein isolates, the conventional isolate showed a 0.09% higher histidine content and a 0.03% higher threonine content.

The amount of arginine in the conventional pea isolate was higher and was closer to the data found in the literature on the amount of arginine in pea powders (Babault et al., 2015). In the literature, the acceptable safe dose of arginine is approximately 20 g/day (McNeal et al., 2016) but the minimum dose that provides a positive effect on health is 2.64 g/day (Sunsandee et al., 2024). Addition of arginine in the diet of rodents – 3.00 g reduced oxidative cell damage, improved mitochondrial function in the brain (Pervin et al., 2021). In a study where serine (0.50g), alanine (1.00g), glutamate (0.50g), aspartate (1.00g) and tyrosine (1.00g) were added to diet of the research group, an improvement in motivation and cognitive functions was observed after 4 weeks (Umeda et al., 2022).

Based on the research, there is no separately determined daily norm of amino acids for patients with psychiatric disorders, so it is important to pay attention to those amino acids that need to be restricted in the diet (Word Health Organization, 2002).

For the pea protein to be absorbed and to have a pleasant taste, it is necessary to evaluate its solution in different pH environments to be able to adjust vegetable or berry juices with different pH concentrations. Consistency is very important for psychiatric patients and in this case, solubility will be determined to understand the application of pea protein in beverage production**.**

To assess the most appropriate processing time and method at which pea protein isolates have the highest solubility, the solubility of the sample was evaluated in an ultrasonic bath and placed in a centrifuge. A significant difference ($p<0.05$) was observed between the samples and the control sample, but there were no significant differences between organic and conventional pea protein isolates. The lowest solubility was observed for those samples treated in an ultrasonic bath for 5 minutes, compared to the control it was higher by 1.38% to 1.39%. The highest solubility was observed in the samples treated for 20 minutes, compared to the control it was higher by 5.45% to 5.46% (Table 3).

In a study in which pea protein isolate was treated with high-intensity ultrasound at different process times (5, 10, and 20 min) powers (200, 300, and 500 W) and amplitudes (40%, 60%, and 100%), it was observed that there was a significant increase in surface charge size and a decrease in particle size with the presence of soluble aggregates. The protein content (1.65 g/25 ml–1.97 g/25 ml) and the absolute solubility of pea protein (66.33–79.92%) improved significantly (Gao et al., 2022). Overall, in the studies, the treatment with high sonication and a time of 20 min significantly improved solubility (Gao et al., 2022).

Table 3

Solubility of organic and conventional pea protein isolates after being treated in an ultrasonic bath set, %, of dissolved protein in water

The time spent in the ultrasonic bath	Organic pea protein isolate %	Conventional pea protein isolate %
Control	8.58	8.58
5 min	9.97	9.96
10 min	11.05	11.02
20 min	14.03	14.04

Table 4

Solubility of pea protein isolate in different pH solutions, %, of dissolved protein in water

pH concentration	Organic pea protein isolate %	Conventional pea protein isolate %
3	53.60	53.32
5	33.80	34.41
7	80.28	80.24
9	59.62	59.64
11	58.32	58.31

Studies have reported that pH can affect the protein solubility, surface hydrophobicity, and protein water retention capacity (Yang et al., 2022), so a study was conducted to analyse the solubility of the pea protein isolate at different pH concentrations at 20 °C. In the study, the data prove that solubility has a significant effect by different pH ($p<0.05$) (Table 4).

In the experiment conducted at a temperature of 20 °C, it was observed that the highest solubility was at pH 7.00, which was 48% higher than at pH 5.00 (Table 4). On the other hand, the solubility at pH 3, 9, 11 was much lower, which could be affected by the temperature of 20 °C (Table 4). This is also confirmed by the data found in other studies, where 65.73% ± 1.64% to 66.48% ± 1.15% solubility was observed at pH 7.00 (Burger et al., 2022), and 23.6% solubility at pH 5.00 (Sun et al., 2023). Several studies have shown that solubility was higher at alkaline pH than at acidic, with the highest solubility observed at pH 12.0 (Sajib et al., 2023). The lowest protein solubility was found at pH 4.0, which is the isoelectric point (4.0–5.0) (de Oliveira et al., 2020). Applying a temperature of 40 °C significantly increased ($p < 0.05$) the protein solubility at pH 9.00 and 12.00 than at 20 °C (Sajib et al., 2023). It is important to emphasise that the solubility of peas is different from the characteristics of the variety and the composition of the protein fractions. Most plant proteins consist of salt-soluble globulins and water-soluble albumins, occurring in proportions of 70% to 20% (Kuang, 2022). Due to the high concentration of globulin in peas, a higher solubility will be observed in a more alkaline environment, as is the case with animal proteins (β-lactoglobulin), where the solubility at pH 7.00 is greater than 80% (Asen et al., 2023). Legumin (11S) and vicilin (7S) are the main types of globulins and their reduced ratio in pea proteins can increase their emulsifying and gelling properties due to their higher protein extractability (Kuang, 2022). The ratio of globulin protein fractions 7S and 11S denaturation temperature is 68 °C and 77 °C, respectively. The ratio between 7S/11S determines denaturation temperature of pea protein isolate, the higher the amount of 11S, the higher the denaturation temperature (Tanger et al., 2020). The solubility of pea protein from pH 6.8 to 7.8 during treatment at 121 °C for 2.8 min showed a high solubility (from 92% to 97%), but at 140 °C for 4 seconds ultra-high temperature (UHT) the solubility decreased significantly, regardless of the pH concentration (Bogahawaththa et al., 2019). Therefore, temperature and pH play a very important role in creating the desired product consistency.

Conclusions

Analysing the amino acid profile, it was found that the most suitable pea protein isolate is organic, as it provides a higher content of total essential amino acids (41.27%), as well as a higher content of leucine, lysine and arginine, which are in line with the doses recommended by the World Health Organization. No significant differences in solubility were found between organic and conventional pea protein isolates, but pH level and sonication time play a significant role in pea protein isolate solubility. The study found significant differences in solubility depending on the duration of the ultrasonic bath and the pH concentration, where it was the highest at pH 7.00 and the lowest at pH 5.00 at a temperature of 20 °C. The obtained data are of fundamental importance in further research in the processing of pea protein isolate.

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