Australian Journal of Crop Science AJCS
Aust J Crop Sci. 20(02):128-132 (2026) | ISSN:1835-2707
https://doi.org/10.21475/ajcs.26.20.02.p94

Analysis of essential and non-essential amino acid composition, total protein concentration and solubility of the protein concentrate extracted from *Avena sativa* L. (white oat)

Gabriela Matte Bertoldi [1], Alana Thais Gisch Andres[1], Karine Raquel Uhdich Kleibert[1], Flávia Alessandra da Silva Räder [1], Viviane Fereira de Mélo[1], José Antônio Gonzalez da Silva[1], Ivan Ricardo Carvalho[1], Raul Vicenzi[1], Luciana Mori Viero[1], Maximiliano Segundo Escalona Jiménez[2], Christiane de Fátima Colet[1]

[1]Northwest Regional University of the State of Rio Grande do Sul (UNIJUÍ). Ijuí, Rio Grande do Sul, Brazil
[2]Dubai Food Industry and Commerce. Ijuí, Rio Grande do Sul, Brazil

Corresponding author: Christiane de Fátima Colet, Department of Pharmacy, Northwest Regional University of the State of Rio Grande do Sul – UNIJUÍ/IJUÍ Campus, Zip Code 98700-000, Ijuí Rio Grande do Sul, Brazil. Email: christiane.colet@unijui.edu.br

Submitted:* *07/08/2025

Revised:* *19/11/2025

Accepted:* *19/11/2025

Abstract: Proteins have important cellular functions. In the search for more functional foods, proteins of vegetable origin stand out as a source of amino acids. The objective of this chapter was to develop an efficient protocol for the extraction and isolation of white oat protein to achieve the technical, sensorial and nutritional quality desired for a functional food, and to characterize its essential and non-essential amino acid composition. This study used white oat bran from the URS Taura cultivar, from which defatted oat protein was extracted using the precipitation method. Amino acid contents were determined using high-performance liquid chromatography. The solubility of oat protein was low and its concentration was 65% protein, characterized by the presence of 18 essential and non-essential amino acids, in with the highest concentrations being leucine (5.08 g/100g) and phenylalanine (4.14 g/100g). These and other important essential amino acids make the present isolated product to compete in content levels with other protein sources such as soy and pea. The amino acids found in lower quantities were methionine (1.05g/100g) and tryptophan (0.1g/100g). The final product presented important content values of essential amino acids. However, some low values indicate the need for association with other protein sources. Further experimental studies are necessary to evaluate the nutritional quality and safety of this product.

Keywords: isolated product; extraction process; leucine; phenylalanine.
Abbreviations: AG_glutamic acid; AA_amino acids; EA_essential amino acids; AS_aspartic acid; ATP_adenosine triphosphate; DAB_4-dimethylaminobenzaldehyde; EAA_sum of essential amino acids; FAO_Food and Agriculture Organization; HPLC_high-performance liquid chromatography; NaCl_sodium chloride; NEAA_sum of non-essential amino acids; P.A._pure for analysis; PITC_phenylisothiocyanate; UV_ultraviolet.

Introduction

The search for new alternatives to meet humanity's essential nutritional needs includes plant-based sources capable of providing nutraceutical food products with added value, considering their rich and distinctive composition (Langyan, 2021). Given the phytochemical composition of oats, consisting of unique primary and secondary metabolites, this cereal represents a promising source for inclusion in nutraceutical products aimed at promoting human health (Silva, Carvalho, & Magano, 2020). The great interest in the use of oats and their components has resulted in a growing number of studies on their chemical composition and the effects of these components as potential agents of pharmacological and nutritional importance (Sandrin, 2013; Malanchen et al., 2019; Battiston, 2017; Silveira, 2012; Kuhn, 2015).
Evidence indicates that oat consumption provides health benefits such as reducing hypercholesterolemia and exhibiting antioxidant activity by inhibiting lipid peroxidation in membranes. These effects are attributed to both soluble fibers (β-glucan) and proteins (Nornberg, Liberali, & Coutinho, 2013; Mira, Graf, & Cândido, 2009; Guimarães, Dadalto, & Figueiredo, 2022; Lásztity, 1998). In addition, oats demonstrate a strong antioxidant capacity related to the presence of aromatic amino acids (approximately 20.93%) (Xu et al., 2013). The proteins of *Avena sativa* stand out not only for their quantity but also for their essential amino acid composition (Silva, Carvalho, & Magano, 2020), including valine, isoleucine, leucine, threonine, histidine, tryptophan, lysine, phenylalanine, tyrosine, and methionine (Biel, Kazimierska, & Bashutska, 2020; Rafique et al.,

2022), representing on average 12–20% of the total protein content. Oats also contain 5–10% lipids, 3–14% total dietary fiber, and 69–76% carbohydrates (Beloshapka et al., 2016; Biel, Jacyno, & Kawęcka, 2014). When compared with other cereals, oats show higher digestibility than wheat and corn (Hernandez, Veja, & Sotello, 1984) and contain amino acids similar to those found in soybeans, which are considered the main plant source of high biological value protein (Mandarino & Panizzi, 2021).

Considering the high protein value of soy, it has important applications and provides all the essential amino acids required by the human body. However, its consumption has been associated with adverse effects related to the presence of phytoestrogens, such as non-progressive precocious puberty, reproductive alterations, and potential genotoxic effects (Fortes et al., 2007; Hollenbach et al., 2010; Klaassen & Watkins, 2012). In this context, oat protein emerges as a safe alternative, as some cultivars exhibit a complete essential amino acid profile (Pedó, Sgarbieri, & Gutkoski, 1999), without the risks attributed to soy.

Studies have already demonstrated the benefits of *Avena sativa*. For example, Xia et al. (2018) conducted a randomized, double-blind, placebo-controlled clinical trial and found improvements in exercise-induced muscle damage. Similarly, recent studies have shown potential antioxidant effects and angiotensin-converting enzyme inhibitory activity in peptides derived from oat protein (Darewicz et al., 2022), as well as promising evidence of digestibility and nutritional value in animal models (Abelilla, Liu, & Stein, 2018). However, despite these benefits associated with oat protein, further research is needed to elucidate the diversity of amino acids in its composition and to ensure the quality of constituent isolation protocols.

In light of the above, the objective of this study is to develop an efficient protocol for the extraction and isolation of white oat protein, aiming to achieve the desired technical and nutritional quality for a functional food, and to characterize its composition in essential and non-essential amino acids.

Results and Discussion

The extraction process resulted in a fine powder of light brown color. Solubility was low, classified as moderately soluble in water and ethyl alcohol, that is, diluted in 30 to 100 parts of the solvent, according to the Brazilian Pharmacopoeia, 6th edition (Brazil, 2022). According to Li and Xiong (2021), solubility is a complex aspect for proteins influenced by their physicochemical formulation, such as molecular size, amino acid composition, and surface hydrophobicity; the side chains of each amino acid (R group) vary, and this variation can increase or decrease solubility, so that an extrinsic factor is necessary to disturb the protein-protein binding. Given this reality, an appropriate ionic strength (salt concentration, in this case, NaCl and Sodium Phosphate) needs to be added in the production of oat protein-containing foods in order to improve protein solubility characteristics (Li and Xiong, 2021; Nelson and Cox, 2014).

The extraction process, performed by the Kjeldahl method, resulted in a white oat protein isolate with a protein concentration of 65%. This value obtained in the present study is lower than that of Rafique et al. (2023), who evaluated the oat protein isolate extracted through a similar method to the one described in the present study, and found a protein concentration of 86.3%. However, Rafique et al. (2023) did not present the protein conversion value, which may contribute to the difference in our results. Pinto et al. (2014) evaluated the protein contents of different protein supplements available in the market from sources other than oats. They found that the protein content of plant-based protein supplements ranged from 64.4% to 74.2%, whereas that of whey protein supplements ranged from 34.5% to 79.3%. They used a conversion factor of 6.25, differing from the one used in the present study, which used 5.83, following Jones (1941).

In view of the above, the white oat's protein isolate in this study showed significant potential for use as a protein supplement. One way to increase the protein levels could be to alter the plant's nitrogen content. Kolchinski and Schuch (2004) explain that the nitrogen absorbed by the plant has a significant influence on determining the protein content of the grain. The availability of this nutrient influences the seed's chemical composition, and increasing nitrogen fertilization dosage raises the protein concentrations in oat kernels without affecting the industrial yield or physiological quality of the seeds.

Besides the protein concentration, the essential and non-essential amino acid composition of an isolate is important for its nutritional quality. Table 1 describes the amino acid composition of the isolate from the present study, as well as data from Gorissen et al. (2018) regarding oat, soy, and pea samples, which are commercially available like f protein sources. As for the tryptophan levels obtained in this study, we found 0.1g/100g of protein; the standards required by the Food and Agriculture Organization (FAO) are 1.4g/100g.

Table 1 shows the superiority of the results obtained for white oat in the present study when compared to Gorissen et al. (2018), and the similarity of our results to those obtained for soybean and pea also (Gorissen et al., 2018). However, all samples are inferior when compared to the standard value set by the FAO. Regarding non-essential amino acids, FAO does not determine reference values. In this study, it is highlighted that Glutamic acid had higher levels in the composition of A. sativa.

As shown in Table 1, the Leucine levels in our study are higher than those required by the FAO and those found by Gorissen et al. (2018) for oat, and similar to those they found for pea and soybean. Additionally, our results align with the values found by Pedó et al. (2018) in three oat cultivars adapted for cultivation in Southern Brazil. In this study, methionine was found to be below the FAO recommendations, but above the value found by Gorissen et al. (2018). Furthermore, as shown in Figure 1, we observe that the FAO does not recommend reference values for histidine, an amino acid in which we observe values of so many 1.5 g/100 g.

Table 1. Content of essential and non-essential amino acids in g/100g isolated from *Avena sativa L.* compared to the results described by Gorissen et al., (2018) and the Food and Agriculture Organization (FAO) standard.

EA	*Avena sativa L.*	*Avena sativa L.*[a]	*Glycine max L.*[a]	*Pisum sativum L.*[a]
Histidina	1.5	0.9	1.5	1.6
Isoleucina	2.9	1.3	1.9	2.3
Leucina	5.1	3.8	5.0	5.7
Fenilalanina	4.1	2.7	3.2	3.7
Lisina	2.1	1.3	3.4	4.7
Metionina	1.0	0.1	0.3	0.3
Treonina	1.8	1.5	2.1	2.5
Valina	3.4	2.0	2.2	2.7
ΣEA	22.0	13.6	19.6	23.5
ANE	*Avena sativa L.*	*Avena sativa L.*[a]	*Glycine max L.*[a]	*Pisum sativum L.*[a]
AS	5.9	-	-	-
AG	15.4	11.0	12.4	12.9
Serina	3.2	2.2	3.4	3.6
Glicina	2.8	1.7	2.7	2.8
Arginina	5.2	3.1	4.8	5.9
Alanina	2.9	2.2	2.8	3.2
Prolina	3.1	2.5	3.3	3.1
Tirosina	3.4	1.5	4.8	2.6
Cistina	2.9	0.4	0.2	0.2
ΣANE	38.9	24.7	31.9	34.4

Legend: "-" when not measured; "ΣEA" sum of essential amino acids; "ΣANE" sum of non-essential amino acids [a] Gorissen et al., (2018); "EA" essential amino acids; "ANE" non-essential amino acid; "AS" Aspartic acid; "AG" Glutamic acid. Source: Author, 2024.

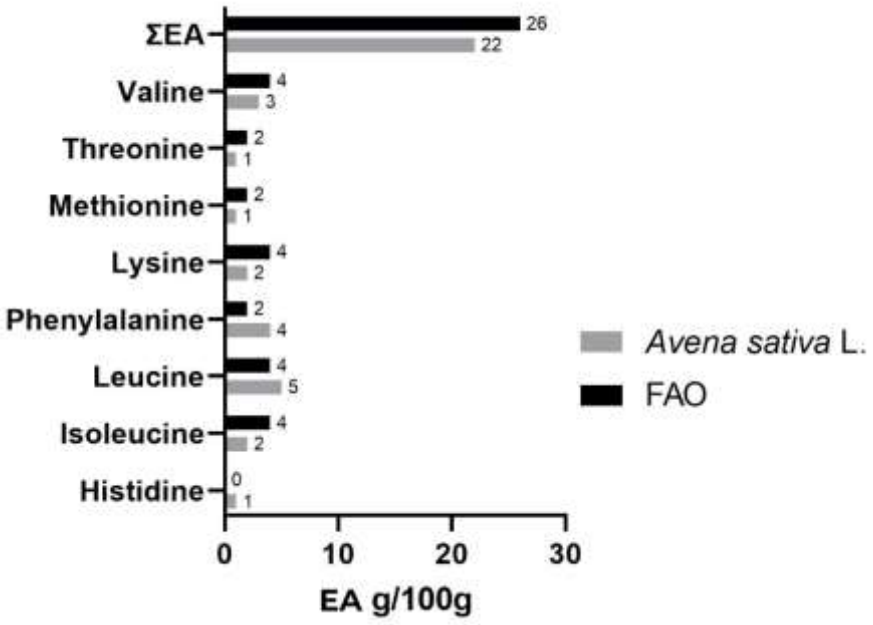

Figure 1. Comparison of amino acids from Avena Sativa L. and Food and Agriculture Organization (FAO) in g/100g. Source: Author, 2024.

An important finding of the present study was the high concentration of leucine. This is a distinctive feature of the extracted oat protein. Leucine is a precursor of ketone bodies, and its degradation has significant implications for ketosis in conditions of prolonged fasting (Nelson and Cox, 2014). Two experimental studies by the same author highlight the benefits of a diet rich in leucine (Cojocaru et al., 2010; Cojocaru et al., 2021). Cojocaru et al. (2010) evaluated the anti-atherogenic effect of leucine by analyzing its effects on the lipid metabolism in laboratory rats in the context of hypercholesterolemia induced by a high-cholesterol diet. The results showed that, when combined with valine, leucine reduced serum cholesterol levels and thus the pro-oxidant state induced by hypercholesterolemia, proving therefore useful in reducing atherosclerosis. In a subsequent study by the same authors, leucine proved useful when in combination with valine to reduce the risk of cardiovascular diseases through a decrease in triglyceride levels. By doing so, the vascular endothelium is protected and the risk of endothelial dysfunction decreases (Cojocaru et al., 2021). Both studies by Cojocaru et al., emphasized that these

two amino acids need to be further explored with respect to their therapeutic potential. The concentrations found in our study hold promise for future food products.

Another study demonstrating the effects of compounds isolated from oat protein on health/disease was conducted by Rafique et al. (2023). They were the first to identify peptides present in oat bran and indicate them as potential neuroprotective agents due to the presence of hydrophobic and aromatic amino acids such as phenylalanine, valine, leucine, alanine, proline, arginine, and tryptophan. Their study found antioxidant properties of the product in an *in vitro* model in cell culture. In a second phase of their study, the peptides were used to improve behavioral performance in zebrafish exposed to scopolamine, resulting in a reduction in acetylcholinesterase (AChE) activity, mitigating oxidative stress and decreasing the levels of inflammatory cytokines in the zebrafish brain. Thus, the study demonstrated that the amino acids found in oat samples have important effects for health in an animal model, calling for further research to explore ways to increase their contents. Another amino acid that presented high content in our study was phenylalanine, with values superior to those found in other species and above FAO recommendations. No experimental studies were found regarding this amino acid, linking its use with health benefits. However, phenylalanine has glucogenic and ketogenic characteristics and can act in two metabolic pathways: in the storage and production of cellular energy (Nelson and Cox, 2014).

In the present study, lysine presented values lower than ~~reduced~~ 50% in relation to FAO recommendation, and was inferior to the content values found in peas and soybeans, which, compared to the results of Gorissen et al. (2018), indicates that it is a characteristic of oats, in presenting less lysine. Abelilla 50% in relation to FAO recommendation, and was inferior to the content values found in pea and soy. Abelilla et al. (2018) indicated that oats have a limited content of lysine, suggesting that this protein needs to be complemented by other plant-based proteins that have higher concentrations of lysine because this amino acid participates in the citric acid cycle, being converted into ketone bodies for use in gluconeogenesis, acting according to physiological needs. Other essential amino acids that have low yield in oats also require attention in terms of supplementation, as amino acids such as methionine, isoleucine, and threonine. These amino acids were found in considerably low levels in the present study. They are glycogenic amino acids, meaning they can be degraded into pyruvate for glucose and glycogen production through metabolic pathways in the production of Adenosine Triphosphate (ATP) in cells (Nelson and Cox, 2014).

Materials and Methods

Plant material

White oat flakes from URS Taura provided by Dubai Indústria e Comércio de Produtos Alimentícios Ltda in Ijuí-RS.

Extraction protocol of Avena sativa L. protein

The methodology described by Rafique et al. (2023), with some adaptations based on the precipitation of protein content, was used to isolate the protein. White oat bran was finely ground and defatted using ethyl alcohol P.A in a 1:4 m/v ratio for 24 hours.

Total nitrogen quantification using the Kjeldahl method

The Kjeldahl method was used to quantify total nitrogen. The results obtained in the titration were applied in the equation, considering the nitrogen-to-protein conversion factor of 5.83 (Jones, 1941).

Analysis of the composition and quantification of amino acids present in the extracted protein isolate

The isolated product was sent to a third-party laboratory for analysis by high-performance liquid chromatography (HPLC) to qualify and quantify the amino acids extracted during the process.

The method used for the quantification of total amino acids is the one described by White et al. (1986) and Hagen et al. (1989). The sample was subjected to acid hydrolysis (vacuum hydrolysis tube, 20 mL, 19 mm x 100 mm) (P/N 29564, Pierce), with pre-column derivatization using phenylisothiocyanate (PITC). The separation of amino acids by HPLC (SHIMADZU®) was performed on a reverse-phase analytical column (LUNA C18 100 A 5 µm 250 x 4.6 mm) (PHENOMENEX®) connected to a C18 pre-column (4 x 3.0 mm) (PHENOMENEX®). The phases used were: mobile phase A [94% sodium acetate buffer 0.0362M (pH 6.4) and 5.7% acetonitrile] and mobile phase B (40% acetonitrile in ultrapure water). A UV detector (SHIMADZU®) was used for identification and quantification of amino acids, an external standard (Pierce / PN 20088) and an internal standard (alpha-aminobutyric acid (Aldrich, Milwaukee-USA) was used, at a wavelength of 254 nm. Analyses were performed in triplicate.

The quantification of tryptophan was determined according to Spies (1967). The sample was subjected to enzymatic hydrolysis with pronase at 40 °C for 22 hours, followed by a colorimetric reaction with 4-dimethylaminobenzaldehyde (DAB) in 21.1N sulfuric acid and read on a spectrophotometer (VARIAN®) at 590 nm. The tryptophan content was calculated from a standard curve of tryptophan (PIERCE®).

For comparison purposes, an article published in the literature was used that presented an analytical methodology for evaluating extracted vegetable proteins similar to the present study (Gorissen et al., 2018).

Conclusion

The final product obtained in this study shows potential in terms of leucine and phenylalanine content values. However, it has considerably low values of other essential amino acids such as lysine, threonine, and methionine, indicating the need for the combined use of this protein product with other protein sources.

In addition to the amino acid content values obtained in this study, we highlight the therapeutic potential of the product, which can be a subject for further investigation.

Acknowledgements

We would like to thank DUBAI Foods for providing the oats and for funding the work and awarding a scholarship.

Conflict of interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

References

Abelilla JJ, Liu Y, Stein HH (2018) Digestible indispensable amino acid score (DIAAS) and protein digestibility corrected amino acid score (PDCAAS) in oat protein concentrate measured in 20- to 30-kilogram pigs. J Sci Food Agric. 98:410–414.
Brasil (2022) Farmacopeia Brasileira, 6th edn, vol 1. Anvisa, Brasília.
Cojocaru E, Leon-Constantin MM, Ungureanu C, Trandafirescu MF, Maştaleru A, Trandafir LM, Petrariu FD, Bădulescu OV, Filip N (2021) Hypolipemiant actions and possible cardioprotective effects of valine and leucine: an experimental study. Medicina. 57:239.
Cojocaru E, Zamfir CL, Lupuşoru CE, Cotuţiu C (2010) The effects of some nonpolar aminoacids—valine, leucine—administration on the arterial wall already exposed to a hypercholesterolemic diet. Rev Med Chir Soc Med Nat Iasi. 114(2):504–509.
Gorissen SHM, Crombag JJR, Senden JMG, Waterval WAH, Bierau J, Verdijk LB, van Loon LJC (2018) Protein content and amino acid composition of commercially available plant-based protein isolates. Amino Acids. 50(12):1685–1695.
Hagen SR, Frost B, Augustin J (1989) Precolumn phenylisothiocyanate derivatization and liquid chromatography of amino acids in food. J Assoc Off Anal Chem. 72(6):912–916.
Jones DB (1941) Factors for converting percentages of nitrogen in foods and feeds into percentages of protein. U.S. Department of Agriculture Circular. 183:22.
Kolchinski EM, Schuch LOB (2004) Relações entre a adubação nitrogenada e a qualidade de grãos e de sementes em aveia branca. Cienc Rural. 34:379–383.
Langyan S, Yadava P, Khan FN, Dar ZA, Singh R, Kumar A (2021) Sustaining protein nutrition through plant-based foods. Front Nutr. 8:734723.
Li R, Xiong YL (2021) Sensitivity of oat protein solubility to changing ionic strength and pH. J Food Sci. 86:78–85.
Nelson DL, Cox MM (2014) Princípios de Bioquímica de Lehninger, 6th edn. Artmed, São Paulo.
Pedó I, Sgarbieri VC, Gutkoski LC (1999) Protein evaluation of four oat (Avena sativa L.) cultivars adapted for cultivation in the south of Brazil. Plant Foods Hum Nutr. 53(4):297–304.
Pinto GMF, Silva KR, Bueno Pereira RF, Sampaio SI (2014) Estudo do descarte residencial de medicamentos vencidos na região de Paulínia (SP), Brasil. Eng Sanit Ambient. 19(3):219–224.
Rafique H, Hu H, Ren T, Dong R, Aadil RM, Zou L, Sharif MK, Li L (2023) Characterization and exploration of the neuroprotective potential of oat-protein-derived peptides in PC12 cells and scopolamine-treated zebrafish. Nutrients. 16:117.
Spies JR (1967) Determination of tryptophan in proteins. Anal Chem. 39:1412–1415.
White JA, Hart RJ, Fry JC (1986) An evaluation of the Waters Pico-Tag system for the amino-acid analysis of food materials. J Autom Chem. 8(4):170–177.
Xia Z, Cholewa JM, Dardevet D, Huang T, Zhao Y, Shang H Yang Y, Ding X, Zhang C,Wang H, Liu S, Su Q, Zanchi NE (2018) Effects of oat protein supplementation on skeletal muscle damage, inflammation and performance recovery following downhill running in untrained collegiate men. Food Funct. 9(9):4720–4729.
Xu C, Lv J, You S, Zhao Q, Chen X, Hu X (2013) Supplementation with oat protein ameliorates exercise-induced fatigue in mice. Food Funct. 4(2):303–309.