Italian Journal of Food Science, 2023; 35 (1): 19–25

Amino acid and fatty acid compositions of texturized vegetable proteins

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Received: 18 August 2022; Accepted: 17 November 2022; Published: 11 January 2023

PAPER

Abstract

Texturized proteins are the main ingredients of meat analogues. This paper evaluated the amino acid and the fatty acid compositions of texturized proteins produced by pea isolates, soy isolates, or dry-fractionated pea proteins, all combined with oat proteins. The nutritional composition was significantly affected by the protein sources. All the texturized proteins had a balanced amino acid profile, complying with the recommendations by FAO/WHO, except for the sulfur amino acids. The fatty acid profile showed the predominance of the polyunsaturated fraction, which was the highest in the dry-fractionated pea mixes. The trans-isomers' content was lower than 0.5%.

Keywords: amino acid composition; dry fractionation; fatty acid composition; meat analogues; plant-based protein; protein concentrates

Introduction

Plant-based meat analogues are gaining considerable interest from both the scientific community and the food industry. For instance, in 2021, the investments in the sector of alternatives to animal proteins reached 5 billion dollars, with a rising trend expected in the next years (GFI, 2021). The shift toward plant-based protein is motivated by consumer awareness about the sustainability of the food chain, which is characterized by the high environmental impact of the animal productions (Espinosa-Marrón *et al.*, 2022), as well as the concerns regarding the adverse health effects associated with the high consumption of red and processed meat products (González *et al.*, 2020).

Texturized vegetable proteins are the main ingredients of meat analogues, and they are commonly produced by the extrusion-cooking process (De Angelis *et al.*, 2020; Dekkers *et al.*, 2018), which is used to give a fibrous texture to the proteins that mimic the structure of the meat muscle.

The production and the characterization of texturized proteins have been previously reported in several studies, in which different protein isolates are commonly used, such as pea and oat (Kaleda *et al.*, 2020); soy and microalgae (Caporgno *et al.*, 2020); soy (Islam *et al.*, 2022); rapeseed (Jia *et al.*, 2021); and soy, mung bean, peanut, pea, and wheat gluten (Samard and Ryu, 2019). However, it should be considered that the protein isolates are extracted and concentrated by using a high resource-demanding process, generally defined as "wet extraction" (Lie-Piang *et al.*, 2021). Since they are almost pure protein ingredients, after the texturization they are added with several other components such as fat, starch, and fiber (Bohrer, 2019; Kyriakopoulou *et al.*, 2021).

In contrast, in our previous study, we demonstrated the possibility of producing the main component of meat analogues (i.e., the texturized vegetable protein) by using dry-fractionated pea proteins (De Angelis *et al.*, 2020). Dry-fractionated proteins are complex ingredients since they have a lower protein content compared to the protein isolates (near 55 g/100 g) but also starch, lipids, and fibers, which are not fully separated during the extraction process. Indeed, dry fractionation is a simple and sustainable technology to produce protein concentrates by solely physical processes (De Angelis *et al.*, 2021a, 2022), and it can be a key strategy for the development of more sustainable and less processed food.

The rapid development of the sector of alternative proteins needs to be supported not only by the studies concerning the texturization process but also by the assessment of the nutritional quality of such products. Indeed, compared to animal protein, vegetable protein sources show a different nutritional value, having a lower content of essential amino acids, and a lower digestibility (Bohrer, 2019; Wu, 2016). Moreover, plant-based meat analogues generally contain high saturated fat, added during the formulation of such products, which is incompatible with the idea of a healthy diet (Hu *et al.*, 2019).

Currently, information about the amino acid composition and the fatty acid profile of the texturized proteins are scarce in the literature, with few references focusing on the protein isolates as ingredients (De Marchi *et al.*, 2021; Kaleda *et al.*, 2020; Samard and Ryu, 2019). This is a concern both for producers and consumers, increasingly interested in these plant-based alternatives to conventional protein sources (Pasqualone *et al.*, 2022).

Therefore, in this paper, we have evaluated the amino acid and the fatty acid compositions of texturized proteins produced by using different protein sources, that is, pea isolates, soy isolates, and dry-fractionated pea, all in combination with oat protein concentrate, produced and characterized for their physicochemical and sensory properties in De Angelis *et al.* (2020).

Material and Methods

Texturized vegetable proteins

The texturized vegetable proteins were produced by using a KETSE 20/40 twin-screw extruder (Brabender GmbH, Duisburg, Germany). The dry-fractionated pea protein – P_{df} – was kindly provided by Innovaprot Srl (Gravina in Puglia, Italy), the pea protein isolates – P_{is} – (Caremoli s.p.a, Monza, Italy), defatted soy protein isolates – S – (Shandong Yuxin Bio-Tech Co., Ltd., Shandong, China), and oat protein concentrates – O – (Lantmännen Oats, Kimstad, Sweden) were provided by the Center of Food and Fermentation Technologies (Tallinn, Estonia). Three protein mixes were tested: (I) P_{df}_O: (70:30 w/w); (II) P_{is}_O: (70:30 w/w); and (III) S_O: (70:30 w/w).

The proximate composition of the mixes is reported in De Angelis *et al.* (2020). As reported in Figure 1, the protein

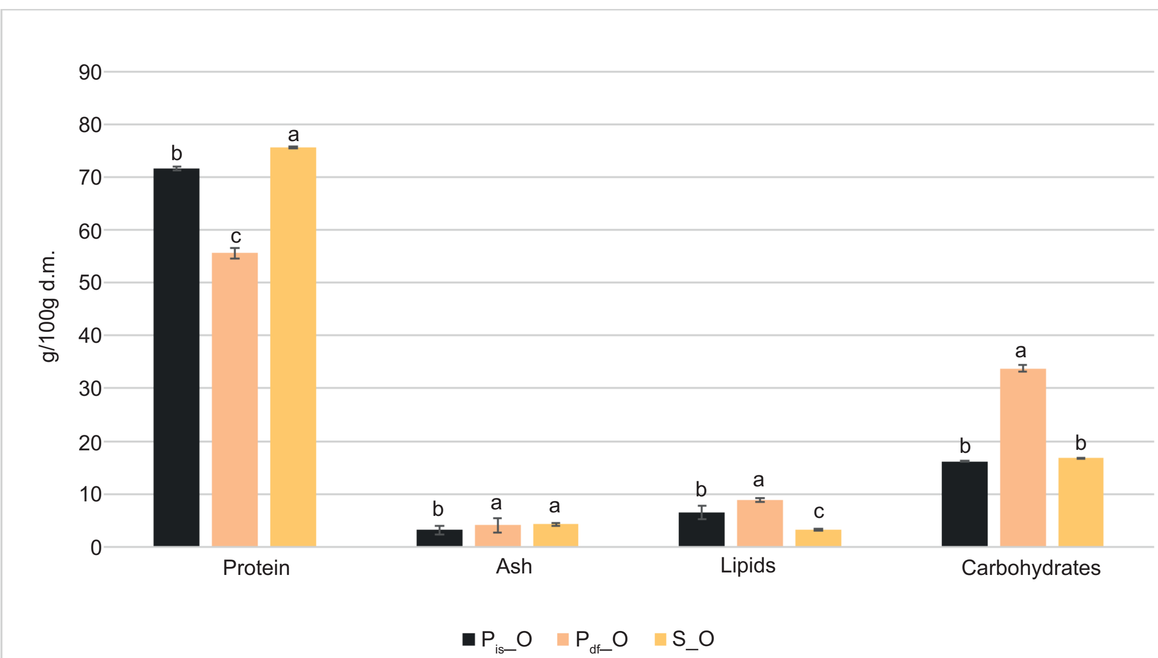

Figure 1. Proximate composition of the texturized proteins according to De Angelis *et al.* (2020). P_{is}_O: Pea isolates/oat protein (70:30 w/w); P_{df}_O: dry-fractionated pea/oat protein (70:30 w/w); S_O: soy isolates/oat protein (70:30 w/w). Data showed as percent mean ± standard deviation on a dry matter basis. n = 3. Different letters for the same parameter mean significant differences at P ≤ 0.05.

content of the products varied from 55 to 75 g/100 g, and the lipid content ranged between 4 and 8.9 g/100 g.

Amino acid composition

The amino acid composition was determined after the acid hydrolysis of the proteins by HCl 6N with 1% phenol (w/v) (Merck KGaA, Darmstadt, Germany) using the Pico-Tag workstation and glass reaction vials (Waters Corporation, Milford, USA). Ten milligrams of sample were dispensed in a glass vial contained in a glass reaction vial. Then, 200 µL of HCl 6N with 1% phenol (w/v) were added to the bottom of the reaction vial and the whole system was closed under nitrogen, to avoid the oxidation of the amino acids. The acid hydrolysis was carried out at 105°C for 24 h.

The hydrolyzed amino acids were recovered with 5 mL of distilled water and then analyzed by high performance liquid chromatography (HPLC), after in-line derivatization with ortho-phthalaldehyde/9-fluorenylmethyl chloroformate (OPA/FMOC) reagents, according to the procedures reported in Henderson and Brook (2010). The HPLC system consisted of an Agilent 1260 equipped with a Diode-Array-Detector (Agilent Technologies, Santa Clara, USA). The column used was a Poroshell 120, HPH-C18, 4.6 × 250 mm, 4 um (Agilent Technologies, Santa Clara, USA). The quantification of the amino acids (expressed as mg/g protein) was carried out with the external calibration curves prepared using amino acid standards at increasing concentrations of 100, 250, and 1000 pmol. The analysis was carried out in triplicate.

Fatty acid composition

The oil was extracted from the texturized proteins by means of an automatic Soxhlet extractor (SER 148 extraction system, Velp Scientifica Srl, Usmate Velate, Italy) using the diethyl ether (Merck KGaA, Darmstadt, Germany) as solvent (AOAC International, 2006). The fatty acid composition was then determined by gas-chromatographic analysis of the fatty acid methyl esters. The methylation was carried out according to the American Oil Chemists Society method Ch 1–91 (AOCS, 1993). The gas-chromatographic apparatus and the conditions of analysis were reported in De Angelis *et al.* (2021b) and were composed of a 7890B gas-chromatograph (Agilent Technologies, Salta Clara, USA) equipped with a flame ionization detector and a fused silica capillary column SP2340 60 m × 0.25 mm × 0.2 µm (Supelco Park, Bellefonte, USA). The identification of each fatty acid was possible by comparing the retention time with that of the methyl ester standards (Merck KGaA, Darmstadt, Germany). The analysis was carried out in triplicate.

The atherogenic (AI) and the thrombogenic (TI) indices were calculated according to Ulbricht and Southgate (1991), using the following equations, considering the content of monounsaturated fatty acid (MUFA), and polyunsaturated fatty acid (PUFA):

$$AI = \frac{C12{:}0 + (4 \times C14{:}0) + C16{:}0}{n-6\ PUFA + n-3\ PUFA + MUFA}$$

$$TI = \frac{C14{:}0 + C16{:}0 + C18{:}0}{0.5 \times MUFA + 0.5 \times n-6\ PUFA + 3 \times n-3\ PUFA + (n-3\ PUFA/n-6\ PUFA)}$$

Statistical analysis

Data were subjected to one-way analysis of variance (ANOVA) followed by the Tukey HSD (Honestly Significant Differences) test for multiple comparisons at a significance level of $\alpha = 0.05$ by using the Minitab 19 Statistical Software (Minitab Inc., State College, PA, USA).

Results

Amino acid composition

The contents of the indispensable and nonessential amino acids expressed as mg/g of protein are reported in Figures 2 and 3, respectively. The protein used as raw material slightly but significantly influenced the amino acid composition of the three mixes under investigation. Regarding the indispensable amino acids (Figure 2), the texturized protein produced with dry-fractionated pea and oat showed the lowest content of the sulfur amino acids – SAA – (methionine + cystine) and aromatic amino acids – AAA – (phenylalanine + tyrosine), whereas no significant differences were identified for histidine, isoleucine, and threonine. The texturized proteins containing dry-fractionated pea and pea isolates then significantly differed only for leucine, SAA, and AAA. The indispensable amino acids are essential for the human body, and they need to be consumed with the diet since they cannot be synthesized by the organism (Herreman *et al.*, 2020). The recommended values for the indispensable amino acid that needs to be consumed with the diet are reported by the experts of FAO/WHO (2013) for different age groups. According to our data, it is possible to highlight that all the texturized proteins matched the values recommended for adults, except for the SAAs, which were slightly lower than that prescribed in all the texturized vegetable proteins, therefore being the limiting amino acid in these products (Reynaud *et al.*, 2021).

To increase the content of methionine and cystine, other protein sources such as hemp, wheat, and rice could be added to the formulation of meat analogues because they are characterized by high values of SAAs (Gorissen *et al.*, 2018).

However, it should be considered that to have a deeper insight into the protein quality, the bioavailability of the proteins should also be evaluated, by measuring the digestible indispensable amino acid score (DIAAS) (FAO/WHO, 2013; Herreman *et al.*, 2020), which is calculated considering the true ileal digestibility of the amino acids. The protein digestibility is largely variable in the studies present in the literature (Herreman *et al.*, 2020), but generally, soy shows the highest digestibility, followed by pea and oat with average values accounting for 91, 70, and 57%, respectively (Herreman *et al.*, 2020). Moreover, it should be considered that the processing technology of proteins, needed to obtain the final food product, causes physicochemical modifications that can affect digestibility, as previously reported by Reynaud *et al.* (2021). This aspect should be treated in future research on plant-based protein foods, in order to highlight the digestibility and protein quality as affected by the protein source and process.

The content of the remaining nonessential amino acids is shown in Figure 3. Significant differences were identified in the content of aspartic acid, glutamic acid, and arginine. By contrast, serine, glycine, and alanine showed no significant differences among the mixes. The pea-based texturized protein showed the lowest content of glutamic acid, whereas the dry-fractionated pea mix contained the lowest content of aspartic acid and arginine.

Although, for nonessential amino acids, an officially recognized recommendation does not exist, other authors had highlighted the necessity to better study even these components (Liu *et al.*, 2019; Wu, 2016). Indeed, the human body in particular conditions, for example, during an illness could not synthesize the nonessential amino acid, compromising protein synthesis (Liu *et al.*, 2019; Wu, 2016). The amino acid composition of the texturized protein satisfied the values recommended in an explorative study carried out by Wu (2016), except for glycine and arginine.

Fatty acid composition

The fatty acid composition of the texturized proteins is reported in Table 1. The products were characterized by the predominance of the polyunsaturated fraction, followed by the monounsaturated one. The PUFA content was the highest in the texturized protein containing pea isolate, whereas the S_O mix showed the lowest content of PUFA, but the highest in MUFA. Small but significant differences were highlighted for the saturated fatty acid content, which was near 20% of the lipid fraction. The three main fatty acids, representative of the three classes were, in decreasing order, linolenic acid, oleic acid, and palmitic acid. The AI and TI significantly changed among the three mixes, and they showed the highest values in the S_O mixes. AI and TI are two indices related to the

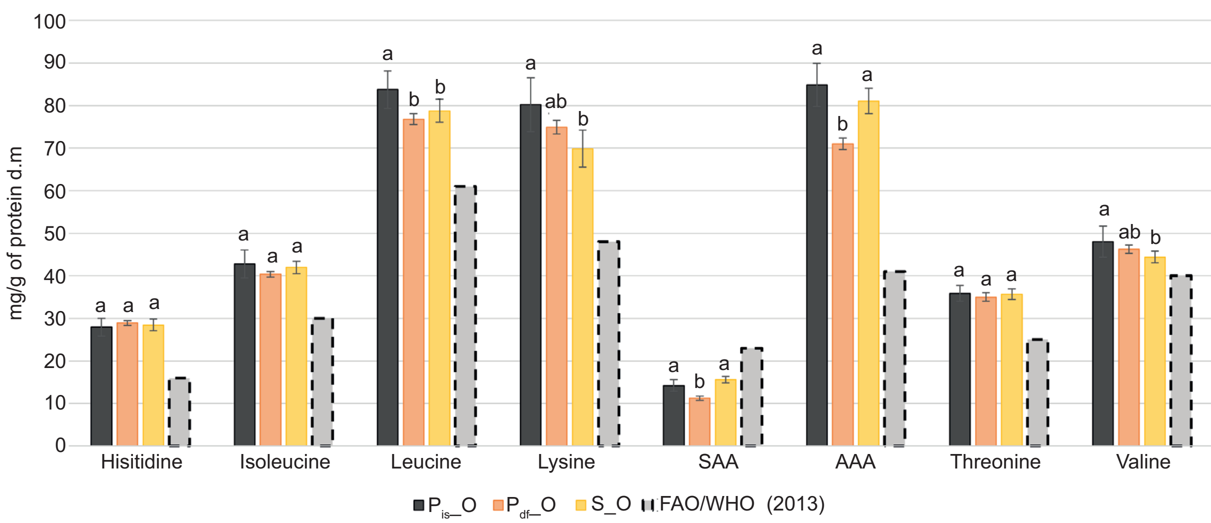

Figure 2. Indispensable amino acid composition of the texturized vegetable protein and recommended values for adults (based on 0.66 g/kg body weight/die) from FAO/WHO (2013). SAA: sulfur amino acids (Methionine + Cystine); AAA: aromatic amino acids (Phenylalanine + Tyrosine). Data showed as mean ± standard deviation. P_{is}_O: Pea isolates/oat protein (70:30 w/w); P_{df}_O: dry-fractionated pea/oat protein (70:30 w/w); S_O: soy isolates/oat protein (70:30 w/w). n = 3. Different letters for the same parameter mean significant differences at P ≤ 0.05.

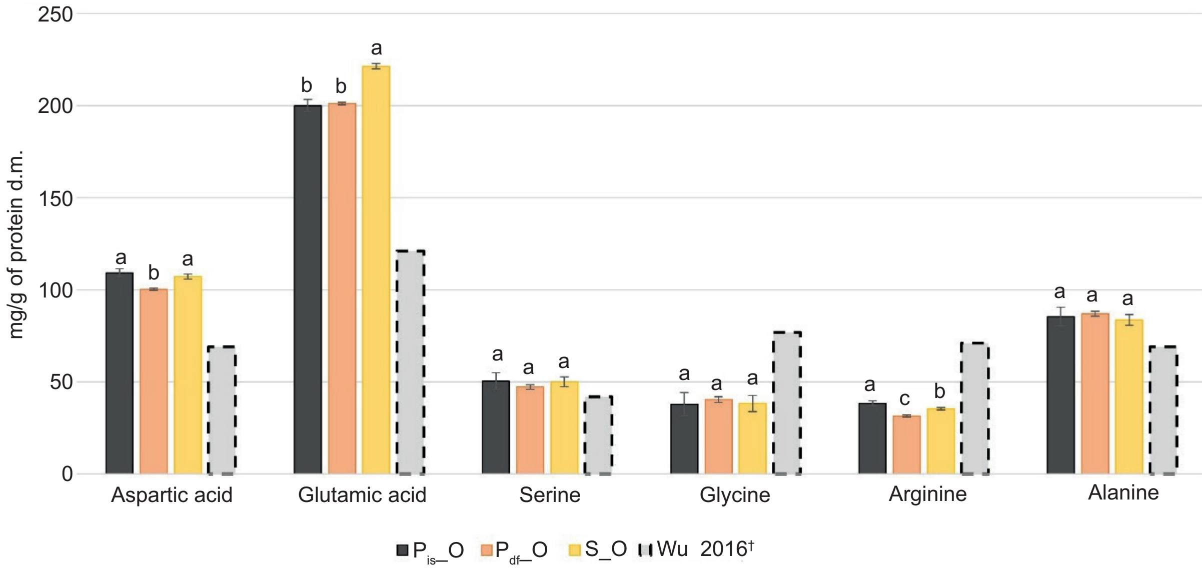

Figure 3. Nonessential amino acids' composition of the texturized vegetable protein. †Recommendations based on the explorative study of Wu (2016). P_{is}_O: Pea isolates/oat protein (70:30 w/w); P_{df}_O: dry-fractionated pea/oat protein (70:30 w/w); S_O: soy isolates/oat protein (70:30 w/w). n = 3. Data showed as mean ± standard deviation. Different letters for the same parameter mean significant differences at P ≤ 0.05.

healthy effect of fatty acids, and lower values are preferable to prevent some coronary diseases (Pleadin *et al.*, 2017). Overall, the fatty acid composition, and the relative indices of atherogenicity and thrombogenicity, was similar to those of other legume species (De Angelis *et al.*, 2021b), suggesting a healthy lipid profile. However, it should be considered that the soy protein is defatted, whereas the oat protein is rich in fat (about 16% as reported in De Angelis *et al.*, 2020). Therefore, the fatty acid composition of S_O was mainly influenced by the oat protein, . Indeed, the fatty acid composition of the soy oat mix reflected previous findings in oat cultivars (Capouchová *et al.*, 2021). In contrast, dry-fractionated pea protein and protein isolates had 4.5 and 3 g/100 g lipids, respectively (De Angelis *et al.*, 2020).

The high content of PUFA is valuable from a nutritional point of view, especially considering that the majority of the meat alternatives available in the market are rich in saturated fats, with consequent concerns related to the health effect of such products (Bohrer, 2019; De Marchi *et al.*, 2021). However, the polyunsaturated fraction can be more susceptible to oxidative phenomena and the development of off-flavors (Velasco *et al.*, 2010), suggesting the need for adequate storage conditions for the texturized proteins.

The total content of the trans-oleic acid was lower in the soy/oat mix compared to the other formulations, whereas the trans-linoleic isomers were constant among the three products. Overall, their quantity was much lower compared to what was previously found in a study conducted by He *et al.* (2021), who reported a content of trans-isomers of the fatty acids ranging between 2.4 and 2.8% of the total. This highlights the fact that there is still a lack of evidence on the health impacts of plant-based food on human health (Wickramasinghe *et al.*, 2021), and the results suggest that promoting the utilization of complex ingredients like the dry-fractionated proteins, containing all the components needed for the final product's formulations, for example, protein, lipids, carbohydrates, and fiber can be a successful strategy not only to improve the nutritional quality of the food but also to reduce the environmental impact of food production.

Conclusions

The amino acid composition of texturized vegetable proteins produced with the combination of pea isolates, soy isolates, and dry-fractionated pea protein with oat protein concentrate was significantly influenced by the specific protein source, but was generally balanced, with the exception of SAAs, with levels lower than that recommended by the FAO/WHO. The nonessential amino acids satisfied the values recommended in an explorative study carried out by Wu (2016), except for glycine and arginine.

The fatty acid profile was characterized by the predominance of the polyunsaturated fraction, and the content of trans-isomers was much lower compared to other commercial products.

Table 1. Fatty acid composition (g/100 g fatty acid) of the texturized vegetable proteins and relative indices.

	P_{is}_O	P_{df}_O	S_O
C14:0	0.43 ± 0.02[b]	0.44 ± 0.05[ab]	0.51 ± 0.02[a]
C15:0	0.07 ± 0.00[a]	0.05 ± 0.00[b]	0.01 ± 0.00[c]
C16:0	14.97 ± 0.22[c]	16.32 ± 0.21[b]	17.52 ± 0.08[a]
C16:1	0.20 ± 0.02[b]	0.21 ± 0.01[b]	0.26 ± 0.01[a]
C17:0	0.09 ± 0.01[a]	0.09 ± 0.01[a]	0.04 ± 0.00[b]
C17:1	0.02 ± 0.00[a]	0.01 ± 0.00[b]	0.01 ± 0.00[b]
C18:0	2.66 ± 0.04[a]	2.28 ± 0.02[b]	1.93 ± 0.02[c]
C18:1t	0.14 ± 0.01[a]	0.12 ± 0.01[a]	0.08 ± 0.01[b]
C18:1	32.02 ± 0.06[c]	33.30 ± 0.28[b]	37.80 ± 0.11[a]
C18:2t	0.28 ± 0.04[a]	0.25 ± 0.03[a]	0.26 ± 0.03[a]
C18:2	42.87 ± 0.06[a]	41.93 ± 0.07[b]	38.84 ± 0.11[c]
C20:0	0.40 ± 0.09[a]	0.37 ± 0.09[a]	0.22 ± 0.02[b]
C18:3	5.61 ± 0.18[a]	4.43 ± 0.07[b]	2.37 ± 0.11[c]
C20:1	0.01 ± 0.00[a]	0.01 ± 0.00[b]	0.00 ± 0.00[c]
C20:2	0.04 ± 0.00[a]	0.03 ± 0.00[ab]	0.03 ± 0.01[b]
C22:0	0.06 ± 0.00[a]	0.04 ± 0.00[b]	0.04 ± 0.00[b]
C23:0	0.04 ± 0.01[b]	0.05 ± 0.01[a]	0.03 ± 0.00[b]
C24:0	0.09 ± 0.00[a]	0.06 ± 0.01[b]	0.03 ± 0.00[c]
SFA	19.71 ± 0.17[b]	18.81 ± 0.25[c]	20.34 ± 0.08[a]
MUFA	32.39 ± 0.08[c]	33.66 ± 0.28[b]	38.16 ± 0.11[a]
PUFA	48.80 ± 0.22[a]	46.64 ± 0.12[b]	41.50 ± 0.19[c]
AI	0.21 ± 0.00[c]	0.23 ± 0.01[b]	0.25 ± 0.00[a]
TI	0.33 ± 0.01[c]	0.37 ± 0.00[b]	0.44 ± 0.00[a]

P_{is}_O: Pea isolates/oat protein (70:30 w/w); P_{df}_O: dry-fractionated pea/oat protein (70:30 w/w); S_O: soy isolates/oat protein (70:30 w/w). SFA: sum of saturated fatty acids; MUFA: sum of monounsaturated fatty acids; PUFA: sum of polyunsaturated fatty acids; AI: atherogenic index; TI: thrombogenic index. Data showed as mean ± standard deviation. n = 3. Different letters for the same parameter mean significant differences at $P \leq 0.05$.

Overall, a deeper assessment of the nutritional value of plant-based protein food is necessary, especially to investigate the protein digestibility and the impact of the formulation of the final meat analogues on the nutritional quality.

Acknowledgments

The authors would like to thank Dr. Luigi Manfredi, InnovaProt Srl and Andriani SPA for providing the raw materials; Aleksei Kaleda, Mari- Liis Tammik, and Martti Tamm from the Center of Food and Fermentation Technologies – TFTAK (Tallinn, Estonia) for the kind support in the extrusion process.

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