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IMPACT OF FOOD PROCESSING ON THE PROTEIN QUALITY AND FUNCTIONAL PROPERTIES OF OILSEED BY-PRODUCTS

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“It is only with the heart that one can see rightly;
what is essential is invisible to the eye…”
(Antoine de Saint-Exupery)

ABSTRACT

In a continuously increasing world population, a current challenge is searching for alternative protein sources, such as plant proteins, with easy supply, low cost, and meeting environmental, social, and sensory aspects. It depends on consumers' preferences, industrial availability, geographical location, and cultural elements. Due to their high protein content, agro-industrial wastes and by-products are potential alternative sources for the human diet. The choice of a protein source greatly depends on its nutritional quality regarding the amino acid profile, bioavailability, and digestibility. The presence of antinutritional factors (ANFs) on a protein source, such as trypsin inhibitors, tannins, and phytates, can significantly influence the protein digestibility, and consequently, protein quality. Food processing may enhance the plant proteins' quality by the inactivation of ANFs, increasing digestibility. Nevertheless, conventional thermal methods may lead to nutritional disadvantages. Thus, emerging technologies with mild process conditions, such as ultrasound and microwave, can produce high-quality products. These techniques can also be used to preserve protein techno-functional properties. This thesis's main objective is to evaluate the impact of food processing, such as cooking, microwave, and ultrasound, on the protein quality of oilseed by-products targeting plant-based protein sources for human nutrition. First, different oilseed by-products from edible oil processing industries were screened, including pumpkin, flaxseed, chia, sesame, and grapeseed. They were characterized by the proximate composition, ANFs, amino acid profile, and in vitro protein digestibility (IVPD). The raw oilseed meals present up to 40% protein content (dry basis) and IVPD between 70 – 85%. In terms of essential amino acid profile, chia seed did not show any deficiency, while the first limiting amino acid in sesame seed and brown flaxseed was lysine and in pumpkin seed, grapeseed, and flaxseed were sulfur amino acids. After the screening, pumpkin seed, flaxseed, and sesame seed meals were processed by cooking, microwave, and ultrasound. Experimental design (central composite) was used to evaluate the influence of processing parameters (temperature, pH, and time) on the IVPD. The best parameters were temperature of 87.8 ºC, pH 8.0, and 37 min, which increased IVPD responses for the oilseed by-products up to 96.1%. Processing was also used to evaluate their impact on ANFs, amino acid composition and score, in vitro protein digestibility-corrected amino acid score (IVPDCAAS), and functional properties (i.e., solubility, water- and oil-holding capacity, and foaming) of the samples. Phytic acid was completely inactivated, and trypsin inhibitory activity decreased up to 84%, while tannins were not detected in the samples. Processing greatly influenced the amino acid composition by reducing some essential amino acids. The first limiting amino acid for all samples was lysine. Nevertheless, cooking, microwave, and ultrasound did not decrease the amino score for the essential amino acids, except for lysine in sesame seed meal, directly affecting the IVPDCAAS. Regarding techno-functional properties, the protein solubility, water- and oil-holding capacity, and foaming properties in the plant matrix demonstrated promising results. Oilseed meals can be used as alternative protein sources for food formulation systems. Therefore, the processes established the potential to increase protein digestibility and eliminate ANFs of oilseed residues. However, further studies are needed to validate these approaches' industrial applications. Finally, proteins from agro-industrial wastes are alternatives for adding commercial value to these products, minimizing negative environmental impacts, and conserving scarce natural resources.

Keywords: Human nutrition; Plant proteins; Agro-industrial by-products; Protein quality; Protein digestibility; Food security.

RESUMO EXPANDIDO

Introdução

Em uma população mundial em crescimento contínuo, um desafio atual é o consumo de proteínas de fácil abastecimento, baixo custo e que atendam aos aspectos ambientais, sociais e sensoriais. As proteínas, como fonte primária de nitrogênio, são macronutrientes indispensáveis para a manutenção da saúde. Encontrar fontes de proteína adequadas é um desafio, pois depende das preferências dos consumidores, disponibilidade industrial, localização geográfica e elementos culturais. Uma fonte de proteína pode divergir em sua qualidade nutricional no perfil de aminoácidos, biodisponibilidade e digestibilidade. A digestibilidade proteica especifica a quantidade de proteína absorvida por um organismo em relação à quantidade consumida e depende da estrutura da proteína, de processamentos prévios e da presença de fatores anti-nutricionais (ANFs), como inibidores de tripsina, taninos e fitatos. A busca contínua por novas e sustentáveis fontes proteicas traz os resíduos agroindustriais como potenciais fontes alternativas para a alimentação humana devido ao seu alto teor proteico. O processamento de alimentos pode melhorar a qualidade proteica das proteínas vegetais, visando aumentar a digestibilidade das proteínas vegetais e a inativação dos ANFs. No entanto, os métodos térmicos convencionais também podem levar a desvantagens nutricionais. Assim, as tecnologias emergentes visam produzir produtos proteicos de alta qualidade, como aquecimento ôhmico, campo elétrico pulsado, alta pressão, ultrassom, plasma frio e processos enzimáticos. Essas técnicas também podem ser usadas para preservar suas propriedades tecno-funcionais. No entanto, poucas avaliações foram feitas sobre o uso de proteínas vegetais, e mais estudos são necessários para validar a aplicação dessas abordagens e sua eficácia para melhorar o valor nutricional das proteínas vegetais. Além disso, embora as tecnologias emergentes tenham grande potencial para preservar as propriedades tecno-funcionais e melhorar a qualidade das proteínas, essas abordagens ainda estão no estágio inicial de suas aplicações industriais. Atender a garantia de uma produção econômica, ecologicamente correta e sustentável para alcançar e ajudar a reduzir os desperdícios e resíduos de alimentos é um desafio determinante para os processamentos emergentes.

Objetivos

O objetivo geral desta tese é avaliar o impacto do processamento de alimentos, como cozimento, micro-ondas e ultrassom, na qualidade da proteína de resíduos agroindustriais provenientes de sementes oleaginosas, visando fontes de proteína vegetal para a nutrição humana. Diferentes sementes oleaginosas das indústrias de processamento de óleo vegetal, incluindo as sementes de abóbora, linhaça, chia, gergelim e uva, foram selecionadas e avaliadas. Estas amostras foram caracterizadas pela composição centesimal, concentração de ANFs, perfil e score de aminoácidos e digestibilidade de proteínas in vitro (IVPD). Após esta triagem e seleção das melhores fontes de proteína (tortas de sementes de abóbora, linhaça e gergelim), os processamentos de cozimento, micro-ondas e ultrassom foram aplicados. O delineamento experimental (composto central) foi utilizado para avaliar a influência de parâmetros independentes de processamento (temperatura, pH e tempo) no IVPD das tortas de sementes selecionadas. O processamento também foi usado para avaliar seu impacto sobre os ANFs, a composição e score de aminoácidos, a digestibilidade proteica in vitro-corrigida pelo escore de aminoácidos (IVPDCAAS) e as propriedades funcionais (solubilidade, capacidade de retenção de água e óleo e formação de espuma) das amostras.

Metodologias
A composição centesimal dos resíduos de sementes oleaginosas foi realizada utilizando procedimentos oficiais da AOAC para umidade, cinzas, lipídios, proteína e conteúdo de fibra. As análises espectrofotométricas de atividade de inibição da tripsina, concentração de taninos e ácido fítico das tortas de sementes foram usadas para medir a concentração de fatores anti-nutricionais. Um método multi-enzimático (tripsina, quimotripsina e pepsina) foi usado para determinar a digestibilidade da proteína in vitro (IVPD) dos resíduos. Cromatografia líquida de alto desempenho (HPLC) foi utilizada para avaliar o perfil de total aminoácidos, enquanto o aminoácido triptofano foi determinado por espectrofotômetro. Com o perfil de aminoácidos das amostras foi possível calcular o escore de aminoácidos e a digestibilidade proteica in vitro-corrigida pelo escore de aminoácidos (IVPDCAAS). Os processamentos de alimentos (cozimento, micro-ondas e ultrassom) foram avaliados sobre a influência dessas tecnologias na qualidade proteica e nas propriedades funcionais (solubilidade, capacidade de retenção de água e óleo e formação de espuma) das amostras.

Resultados e Discussão
Em relação ao *screening* das tortas de sementes de abóbora, linhaça, chia, gergelim e uva, os resíduos apresentaram até 40% de proteína e 70 – 85% de IVPD. Para o perfil de aminoácidos essenciais, a semente de chia não apresentou nenhuma deficiência, enquanto o primeiro aminoácido limitante na semente de gergelim e linhaça marrom foi a lisina; na semente de abóbora, uva e linhaça dourada foram os aminoácidos sulfurados. Após este *screening*, sementes de abóbora, linhaça e gergelim foram selecionadas para serem processadas por cozimento, micro-ondas e ultrassom. A partir do delineamento experimental, os melhores parâmetros foram 87,8 ºC de temperatura, pH 8,0 e 37 min de tempo de processamento, onde aumentaram o IVPD para os resíduos em até 96,1%. O ácido fítico foi completamente inativado e a atividade inibitória da tripsina diminuiu em até 84%, enquanto os taninos não foram detectados nas amostras. O perfil de aminoácidos foi determinado tanto para as sementes cruas e processadas, e o primeiro aminoácido limitante para todas as amostras foi a lisina. O processamento influenciou muito a composição de aminoácidos, reduzindo alguns aminoácidos essenciais. Porém, o cozimento, o micro-ondas e o ultrassom não diminuíram o escore de aminoácidos para os aminoácidos essenciais das amostras, exceto para a lisina nas tortas de semente de gergelim, afetando diretamente o IVPDCAAS de amostras de sementes de gergelim processadas. Em relação às propriedades tecno-funcionais, a solubilidade proteica, a capacidade de retenção de água e óleo e as propriedades de formação de espuma na matriz vegetal para amostras processadas demonstraram que estes resíduos são fontes promissoras e podem ser utilizadas como alternativas proteicas em formulação de alimentos.

Considerações Finais
Cozimento, micro-ondas e ultrassom são métodos de processamento promissores que demonstraram potencial para aumentar a digestibilidade proteica de proteínas vegetais e reduzir os fatores anti-nutricionais de resíduos de sementes oleaginosas. Portanto, as proteínas de resíduos agroindustriais são ótimas alternativas para agregar valor comercial a esses subprodutos, minimizando os impactos ambientais negativos e conservando os recursos naturais.

Palavras-chave: Nutrição humana; Proteínas vegetais; Resíduos agroindustriais; Qualidade proteica; Digestibilidade proteica in vitro; Segurança de alimentos.

LIST OF FIGURES

LIST OF TABLES

SUMMARY

CONCEPTUAL DIAGRAM

Why?

- Food security and the challenge of a growing world population encourage the search for alternative protein sources.
- The use of alternative sources of protein meets the Sustainable Development Goals of the ONU 2030 Agenda, which sets the goals of “Zero hunger" (2), "Good health and well-being" (3), "Responsible consumption and production" (12), and "Climate action" (13).
- Processing can improve the protein quality of plant proteins by inactivating the antinutritional compounds and increasing protein digestibility while enhancing the protein techno-functional properties.
- Oilseed by-products from the oil extraction industries can be sustainable and high-quality protein sources, which may be used as technological ingredients for food formulations and may become extra income for the industry while minimizing large waste disposals and collaborating with the environment.

What has been done?

- Several plant sources have been widely studied and used as protein supplements, such as legumes, cereals, pseudocereals, seeds, almonds, and nuts.
- There are many studies about applying thermal processing on plant proteins aiming to improve their quality, such as cooking, autoclaving, germination, irradiation, drying, and extrusion.
- Few reports are available in the literature on the utilization of non-thermal emerging techniques, such as ultrasound, high pressure, cold plasma, and enzymatic processes to improve the protein quality and functional properties of plant proteins.
- The literature is scarce on the use of emerging methods to evaluate protein digestibility and amino acid composition of plant proteins by-products.

Hypotheses

- The improvement of protein digestibility of plant proteins and by-products using processing is possible.
- High-quality proteins for human nutrition can be obtained from agro-industrial residues.

Methodologies

- Proximate composition of the oilseed by-products using official AOAC procedures for moisture, ash, lipids, protein, and fiber content.
- Analysis of trypsin inhibition activity, tannin, and phytic acid content of the samples to measure the concentration of antinutritional factors.
- A multi-enzyme essay method was used to determine the in vitro protein digestibility.
- High-performance liquid chromatography evaluated the total amino acid profile, while tryptophan was spectrophotometrically determined.
- Processing (cooking, microwave, and ultrasound) was applied to evaluate the influence of these technologies on the nutritional quality and functional properties of the samples.

Responses

- Oilseed by-products as sustainable alternatives for plant-based high-quality protein sources, regarding protein digestibility, essential amino acid profile, antinutritional factors, and techno-functional properties.

CHAPTER I

1 INTRODUCTION

Proteins are essential macronutrients as structural and functional components to maintain humans' growth and other physiological functions. They supply amino acids, which are building blocks in the human body and the main nitrogen source in the human diet (BOYE; WIJESINHA-BETTONI; BURLINGAME, 2012; ADENEKAN et al., 2018). Protein also performs relevant functional roles in food formulation, processing, storage, and consumption, benefiting the sensory and quality attributes (DAY, 2013; MIRMOGHTADAIE; ALIABADI; HOSSEINI, 2016).

Nowadays, food security is a primary challenge for humankind. The demand for protein supply has increased due to the rising world population (about 10 billion by 2050) and the limited environmental resources (NADATHUR; WANASUNDARA; SCANLIN, 2016; BERRYMAN et al., 2018; POJIĆ; MISAN; TIWARI, 2018). Traditional animal proteins are associated with high production costs and negative environmental impacts to livestock farming, such as climate change, freshwater depletion, and biodiversity losses (SUN et al., 2012; ZHAO et al., 2014; ALEMAYEHU; BENDEVIS; JACOBSEN, 2015; ADENEKAN et al., 2018). This encourages the search for sustainable and environmentally feasible high-nutritional foods, including exploring alternative protein sources, requiring developing techniques to evaluate and increase their nutritional quality (SUN-WATERHOUSE; ZHAO; WATERHOUSE, 2014).

Protein quality refers to protein digestibility, amino acid profile, and bioavailability. It is an important criterion for adequate nutrition and maintenance of good health (BOYE; WIJESINHA-BETTONI; BURLINGAME, 2012). Protein digestibility indicates the absorbed protein amount by the organism relative to the protein consumption, and it affects the protein requirements in the human diet (LÓPEZ et al., 2018). It depends on their protein structure, the presence of some compounds that are prejudicial to protein digestion (antinutritional factors), and thermal processing intensity (MATTILA et al., 2018). Therefore, determining the food protein capacity to satisfy metabolic demands for amino acids and nitrogen is an important aspect of protein quality evaluation (HAN; CHEE; CHO, 2015; PENCHARZ; ELANGO; WOLFE, 2016).

There are different ways to determine protein digestibility. Methods frequently used for nutritional quality assessment and determination of in vivo protein digestibility, include

protein efficiency ratio (PER), net protein ratio (or retention) (NPR), net protein utilization (NPU), biological value (BV), true digestibility (TD), protein digestibility-corrected amino acid score (PDCAAS) and digestible indispensable amino acid score (DIAAS) (BOYE; WIJESINHA-BETTONI; BURLINGAME, 2012; MATHAI; LIU; STEIN, 2017). These methods are different, and the results are not directly comparable, but all can be used to indicate the protein quality of a protein source. However, the bioassays with animals to determine digestibility are expensive and time-consuming procedures. Then, in vitro methodologies have been developed in the last century (LÓPEZ et al., 2018). It can be designed to use specific enzymes to give maximal digestibility values and measure hydrolysis's initial rate. The applicability of the results depends on a high correlation with in vivo values obtained under standardized conditions. Concerning the in vitro protein digestibility-correct amino acid score (IVPDCAAS), some authors suggested that this approach could be used as an alternate method for assaying protein quality that does not rely on animal experimentation (NOSWORTHY et al., 2017a).

An increasing global trend for plant-based diets (KYRIAKOPOULOU; DEKKERS; VAN DER GOOT, 2019; STONE et al., 2019) and the use of new protein sources have been recent, including fungi, algae, insects, as well as wastes from food processing, which can meet the higher protein need in the human diet (BOLAND et al., 2013; ADENEKAN et al., 2018; CONTRERAS et al., 2019). Several plant sources have been widely studied and used as protein supplements, such as legumes (CODA et al., 2017; TUŚNIO et al., 2017), cereals, pseudocereals (LÓPEZ et al., 2018), seeds (MATTILA et al., 2018), almonds, and nuts (SOUSA et al., 2011).

Finding sources with protein quality similar to animal ones and developing novel food processing techniques to enhance the traditional plant protein sources nutritional quality are the main challenges in this field. The development of delicious, nutritious, healthy, affordable, and convenient alternative protein products for consumers' acceptance is the target regarding cultural and sensory attributes (e.g., appearance, taste, texture, and flavor).

Although plant proteins are valuable in the human diet, they are regularly recognized as nutritionally inferior or incomplete to animal proteins (HUGHES et al., 2011) due to some deficiency in the essential amino acid composition and the presence of the antinutritional factors, such as trypsin inhibitors, tannins, and phytates (MULTARI; STEWART; RUSSELL, 2015). The elimination of these compounds is imperative to improve the biological utilization of plant proteins, which often have lower digestibility (75 – 80 %) when compared to animal

ones (90 – 95 %) (such as meat, poultry, egg, and milk) (KNISKERN; JOHNSTON, 2011; ANNOR et al., 2017). Thus, researchers point that thermal techniques can improve the plant protein nutritional quality and eliminate these compounds (BOYE; WIJESINHA-BETTONI; BURLINGAME, 2012), such as cooking (KAMELA et al., 2016), autoclaving, germination (KALPANADEVI; MOHAN, 2013), irradiation (MECHI; CANIATTI-BRAZACA; ARTHUR, 2005), drying (TANG, 2007), and extrusion (WU et al., 2015).

Conventional food processing methods can lead to disadvantages, such as high time- and energy-consuming procedures (prolonged heating and stirring), large amounts of water, and losses of desirable compounds (CHEMAT; HUMA; KHAN, 2011). Emerging technologies have been investigated and contribute to environmental preservation, shorten the extraction time, and reduction of wastewater and organic solvents (GOLBERG et al., 2016), such as ultrasound, pulsed electric energy, high pressure, ohmic heating, cold plasma, and enzymatic processes (RUIZ, 2016; POJIĆ; MISAN; TIWARI, 2018; AL-RUWAIH et al., 2019).

Furthermore, plant protein sources from industrial by-products have been stimulated by the sustainability concept in the food processing industry (POJIĆ; MISAN; TIWARI, 2018). The plant proteins from food by-products are ideal for new alternative protein sources regarding sustainability and carbon footprint. Oilseed meals (press cakes) are the most valuable by-products from edible oil extraction industries due to their high protein content (up to 50%) after the oil extraction from the seeds (SARKER et al., 2015; PETRUSÁN; RAWEL; HUSCHEK, 2016). Often discarded or conventionally used as fertilizer and feedstock for animal feed, these by-products have valuable compounds, such as anthocyanins, carotenoids, and polyphenols that can be recovered and used as functional additives in different pharmaceutical and food products (GOLBERG et al., 2016).

Few studies evaluated oilseed by-products proteins as perspective sources of protein for consumption and human nutrition, such as flaxseed, sesame seed (TERRIEN, 2017), rapeseed (or canola) (VOUDOURIS et al., 2017), sunflower seed (DAY, 2013), pumpkin seed (EL-ADAWY; TAHA, 2001), grapeseed (FANTOZZI, 1981; KAMEL; DAWSON; KAKUDA, 1985), cottonseed, peanut (TERRIEN, 2017), and mustard seed (SARKER et al., 2015).

In this scenario, combined with the growing search for the development of new and more efficient processes using less energy, the emerging technologies cited above become the focus of the attention of many studies. Thus, the protein utilization from alternative sources using these new approaches is interesting for industrial applications, making it possible to add

value to renewable raw materials, such as agro-industrial by-products. Based on these issues, this work aimed to investigate the potential of oilseed by-products as high-quality protein sources since scarce studies were found in the literature evaluating their nutritional value. Therefore, this thesis can contribute to the environment by reducing waste disposal and expanding the range of options in utilizing sustainable sources for human consumption.

1.1 OBJECTIVES

1.1.1 General objective

This thesis evaluated the processing of oilseed by-products (pumpkin seed, flaxseed, and sesame seed meals) by cooking, microwave, and ultrasound, targeting to assess their nutritional quality in terms of digestibility, amino acid profile, antinutritional factors and their techno-functional properties looking for plant-based protein sources for human nutrition.

1.1.2 Specific objectives

- Screening of different oilseed by-products from press-cold extraction, such as pumpkin seed, flaxseed, chia seed, sesame seed, and grapeseed, by the characterization of the proximate composition and protein quality evaluation;
- Evaluate the concentration of antinutritional factors (ANFs) by trypsin inhibitor activity, tannins and phytic acid content;
- Evaluate the in vitro protein digestibility (IVPD), amino acid (AA) profile, AA score, and in vitro protein digestibility-corrected amino acid score (IVPDCAAS) of the oilseed meals;
- Evaluate the impact of food processing on the ANFs, AA profile and score, IVPD, and IVPDCAAS of the selected oilseed by-products;
- Evaluate the impact of food processing on the functional properties (solubility, water- and oil-holding capacity, and foaming) of the selected oilseed meals.

CHAPTER II

2 LITERATURE REVIEW

The literature on the pertinent subjects of this thesis is presented in this chapter. Firstly, important information about traditional animal proteins, plant protein sources, and nutritional value are introduced. The evaluation of conventional and emerging processes' impact on protein quality is also described. Finally, the utilization of alternative protein sources current state-of-the-art concerning agro-industrial residues is presented.

This literature review chapter is based on articles already published by the author. Article A, "Food processing for the improvement of plant proteins digestibility", is available in ***Critical Reviews in Food Science and Nutrition*** journal (SÁ; MORENO; CARCIOFI, 2019). The review article B, entitled "Plant proteins as high-quality nutritional source for human diet", was published in ***Trends in Food Science & Technology*** (SÁ; MORENO; CARCIOFI, 2020). Furthermore, article C, "Influence of emerging technologies on the protein digestibility and techno-functional properties of plant proteins", published in ***Frontiers in Nutrition*** (SÁ et al., 2022).

2.1 TRADITIONAL PROTEIN SOURCES

Meat, poultry, egg, and milk, are the main sources of animal protein worldwide. Animal sources have high protein quality regarding protein digestibility and amino acid composition and have sensory characteristics the consumer seeks, such as taste, appearance, and texture. Animal protein consumption, especially meat, is related to cultural aspects, eating habits, and traditions. The meat consumption will not end, but it is important to rethink this high intake due to environmental issues, including freshwater use, climate change, land-use change, and biodiversity loss (GRASSO et al., 2019). Moreover, consumers are concerned about animal welfare (GAVELLE et al., 2017; HARTMANN; SIEGRIST, 2017). In terms of carbon footprint, meat from extensive production systems show by far the largest carbon footprints per kg edible product, while plant products have the smallest impacts (NIJDAM; ROOD; WESTHOEK, 2012). This concern about the environmental impact of the livestock farming and meat industry motivates the search for dietary strategies to increase protein intake, nutritious alternative sources, and the development of processes for reaching the required

protein quality for the plant sources. World's population is increasing continuously, and it is difficult to guarantee food security based on Earth's limited resources and economic constraints. Thus, it is imperative to find intelligent alternatives to meet the nutritional needs of humankind, valuing the environment, cultural aspects, and people's dignity. It has been demonstrated that proteins from plant sources are abundant and widely found, with a potential nutritional profile. Several well-known sources of plant proteins may supply the human diet and help overcome the population growth challenge (WANG et al., 2010; HUGHES et al., 2014).

Traditional plant protein sources have been used, such as soybean, beans, and pea. Studies have shown that different sources can lead to a high-quality protein, including legumes (e.g., chickpea, fababean, pigeon pea, and lupin) (WANG et al., 2010; MATTILA et al., 2018), cereals (e.g., rice, barley, and millet) (AMAGLIANI et al., 2017), pseudocereals (e.g., quinoa, amaranth, and buckwheat) (LÓPEZ et al., 2018; MATTILA et al., 2018), seeds (e.g., flaxseed and chia seed) (OLIVOS-LUGO; VALDIVIA-LÓPEZ; TECANTE, 2010; GIACOMINO et al., 2013), leaves (e.g., *ora-pro-nóbis*) (TAKEITI et al., 2009), and nuts (e.g., peanut and cashew nut) (SOUSA et al., 2011). However, the same plant protein can vary in composition (e.g., protein, oil content, and amino acid profile), according to differences of climatic and soil diversity, geographic altitude and latitude, precipitation levels, agricultural practices, and varietal/cultivars (SUN et al., 2012; LIU; ZHENG; CHEN, 2017).

Plant proteins consumption is associated with a significant decrease in cardiovascular diseases (CVD), low-density lipoprotein (LDL) cholesterol, obesity, and type II diabetes mellitus due to the composition of polyunsaturated fatty acids, fiber, oligosaccharides, and carbohydrates; while ingesting high amounts of animal-based protein tends to increase these health issues risk due to its lipidic profile (GUASCH-FERRÉ et al., 2019a). Furthermore, red meat intake is greatly associated with saturated fats consumption. Therefore, protein diversification from plant foods may contribute to healthier aspects of the human diet, reducing the chances of cardiovascular and chronic diseases (GUASCH-FERRÉ et al., 2019b).

Consumers present growing interest in plant proteins replacing animal ones, while food companies are working on improving the nutritional value of their products. In this scenario, plant protein isolates may be an economical alternative to enrich formulations, incorporating new and unconventional protein sources available in large quantities in some regions. Besides, protein isolates are excellent dietary supplements and functional ingredients because they are the purest protein form (HAN; CHEE; CHO, 2015; ADENEKAN et al., 2018).

2.2 ALTERNATIVE PROTEIN SOURCES

New protein sources, including fungi, algae, insects, and wastes from food processing, can meet the higher protein need in the human diet (CONTRERAS et al., 2019). Currently, the agri-food industry generates 190 million tons of by-products worldwide per year, including pomaces, leaves, peels, seeds, brans, and oilseed meals, and requires waste management, disposition, and recycling (KUMARI et al., 2018; GENÇDAĞ; GÖRGÜÇ; YILMAZ, 2020).

Valuable compounds can be recovered from food by-products, such as proteins, lipids, carbohydrates, phenolics, dietary fibers, and pigments, which can benefit the global food sustainability, economy, and environment (GENÇDAĞ; GÖRGÜÇ; YILMAZ, 2020; KUMAR et al., 2021). Oilseeds have great potential as an economic source of fatty acids and bioactive metabolites. Studies demonstrated the presence of significant amounts of carotenoids, phenolic compounds, tocopherols, and phytosterols in pumpkin seeds (VERONEZI; JORGE, 2012; RABRENOVIĆ et al., 2014); linolenic and linoleic acid, and lignans in flaxseeds (SHIM et al., 2014); polyunsaturated fatty acids and tocopherols in chia seeds (GAHFOOR et al., 2018); phytosterols, polyunsaturated fatty acids, tocopherols, and lignans (e.g., phenylpropanoid compounds) in sesame seeds (PATHAK et al., 2014); linolenic and linoleic acid, tocopherols, and catechins in grapeseeds (AL JUHAIMI; ÖZCAN, 2018). The use of agricultural food by-products is a feasible alternative that increase limited sources of bioactive compounds and non-animal proteins (POJIĆ; MISAN; TIWARI, 2018). These by-products may be called health foods which provide health benefits to consumers and can be used as food supplements because of their nutrients (SUNIL et al., 2016).

Through the extraction of lipids from plant seeds, the edible oil industry yields a high quantity of defatted residue containing an outstanding quantity of proteins and fibers. They can feasibly used as functional ingredients for human nutrition, increasing the value of these by-products (SUN-WATERHOUSE; ZHAO; WATERHOUSE, 2014; MATTILA et al., 2018). For example, considering the annual production of sesame seeds in the world (~6 million tons), approximately 18% of the total weight is separated as industrial by-products (~1 million tons) (GÖRGÜÇ; BIRCAN; YILMAZ, 2019). Since sesame seed meal contains approximately 33% protein (SÁ et al., 2021), about 330,000 tons of plant-based protein from sesame seeds by-products can be recovered annually.

Table 1 summarizes the principal agro-industrial by-products from the edible oil industry aiming the utilization as a protein source.

Table 1 – Food by-products from the edible oil industry evaluated as a protein source.

Potential proteins from oil processing by-products	Protein content (%)	Protein digestibility (%)	References
Soybean	45 – 49	85	(BOLAND et al., 2013)
Corn	5.5 – 8	*	(ONWULATA; KONSTANCE, 2006; CARVALHO et al., 2010)
Rapeseed (or canola)	35.7 – 50	79.5 – 91.4	(MANSOUR et al., 1993a; AIDER; BARBANA, 2011; PETRUSÁN; RAWEL; HUSCHEK, 2016; ZHANG et al., 2017; MATTILA et al., 2018)
Sunflower seed	20 – 40	95.4	(RAYMOND; INQUELLO; AZANZA, 1991; CONDE et al., 2005; SALGADO et al., 2012; SUN-WATERHOUSE; ZHAO; WATERHOUSE, 2014)
Cottonseed	30 – 42	78	(BOLAND et al., 2013)
Sesame seed	21.8 – 47	86.3	(MANTOANI; PESSATO; TAVANO, 2013; PETRUSÁN; RAWEL; HUSCHEK, 2016)
Pumpkin seed	26.6 – 36.5	61.8 – 94.7	(EL-ADAWY; TAHA, 2001; GIAMI, 2004)
Flaxseed	35 – 42.9	64.1 – 72.7	(BARTKIENE; JUODEIKIENE; VIDMANTIENE, 2012; MARAMBE; SHAND; WANASUNDARA, 2013; PETRUSÁN; RAWEL; HUSCHEK, 2016)
Grapeseed	8.2 – 10	77	(FANTOZZI, 1981; KAMEL; DAWSON; KAKUDA, 1985; DING et al., 2018)
Black mustard seed	38.2	80.3	(SARKER et al., 2015)
Yellow mustard seed	28.8	77.4	(SARKER et al., 2015)
Peanut	50 – 55	92.7 – 94	(SOUSA et al., 2011; ZHAO; CHEN; DU, 2012; HE et al., 2014; ARYA; SALVE; CHAUHAN, 2016)
Hazelnut	39 – 43	*	(BILGIN; TÜRKER; TEKINAY, 2007; BUYUKCAPAR; KAMALAK, 2007)
Coconut	4 – 25	*	(RODSAMRAN; SOTHORNVIT, 2018)

* Data not found in the respective study.

The recovery of protein from agro-industrial by-products and residues from the food industry is a sustainable alternative to minimize waste disposal, maximize resources, and add market value to different products. Also, it contributes to developing nutritional products with reduced cost (SALGADO et al., 2012; SUN-WATERHOUSE; ZHAO; WATERHOUSE, 2014). The assessment of sustainable protein resources, including the agro-industrial discarded by-products and wastes, is a great perspective in this field. However, scarce studies can be found in the literature correlating these by-products, the nutritional composition, and the prospect of their consumption in human nutrition.

Finding the most appropriate protein source is challenging. There is no simple way to do it. The choice depends on many aspects, such as geographical location, cultural elements, harvesting and production costs, industrial availability, the scale of production, processing technologies, and consumers' preference. On the other hand, one can select a potential plant protein source using a direct comparison of the amino acid profile of each source to reference patterns, identifying those that have sufficiency in the essential amino acids. More information about the nutritional composition of traditional and alternative plant protein sources can be found in Sá; Moreno; Carciofi (2020).

2.3 PROTEIN QUALITY OF PLANT PROTEINS

Protein quality is the protein capacity to replace the nitrogen that the organism inevitably loses due to the metabolism in the biological processes (AGUILAR et al., 2015). It depends not only on the ingested protein amount but also on age, health status, physiological status, and energy balance. Moreover, the protein digestibility and the presence and bioavailability of essential amino acids are the principal criterion for protein quality, leading to growth and health maintenance in humans (ARRIBAS et al., 2017). There are some methods to evaluate protein quality. Table 2 summarizes the methods frequently used for the determination of in vitro and in vivo protein digestibility.

The amino acid profile of plant protein sources demonstrated their appealing nutritional quality and potential for human nutrition. However, depending on the source, plant proteins may be deficient in some essential amino acids. Although cereals usually contain low levels of lysine and legumes have a deficiency in sulfur amino acids (methionine and cysteine) (VENDEMIATTI et al., 2008; NOSWORTHY et al., 2017b), pseudocereals (e.g., amaranth and quinoa) are good sources of lysine (ALVAREZ-JUBETE; ARENDT; GALLAGHER, 2010).

Table 2 – Methods frequently used for determination of in vitro protein digestibility and in vivo protein quality (SÁ; MORENO; CARCIOFI, 2019).

Protein quality evaluation methods	Observations	Calculation	References
In vitro			
IN VITRO PROTEIN DIGESTIBITY IVPD	Estimation of protein quality; Rapid and low-cost procedure; Overestimates the true nutritional value, since it disregards the concentration and availability of amino acids;	Akeson and Stahmann (1964) ➔ pepsin and pancreatin $IVPD\ (\%) = \frac{\text{Digested protein}}{\text{Total protein}}.100$ Hsu et al. (1977) ➔ trypsin, chymotrypsin and peptidase $IVPD\ (\%) = 210.464 - 18.103.pH_{10min}$	(AKESON; STAHMANN, 1964; RAMACHANDRA; MONTEIRO, 1990; MANSOUR et al., 1993a; BISHNOI; KHETARPAUL, 1994; YADAV; KHETARPAUL, 1994; PREET; PUNIA, 2000; MARPALLE et al., 2015; CODA et al., 2017) (HSU et al., 1977; CLEMENTE et al., 1998; SÁNCHEZ-VIOQUE et al., 1999; HABIBA, 2002; LQARI et al., 2002; SHIMELIS; RAKSHIT, 2005; WANG et al., 2008; PARK; KIM; BAIK, 2010; PASTOR-CAVADA et al., 2010; ALETOR, 2012; BARTKIENE; JUODEIKIENE; VIDMANTIENE, 2012; SALGADO et al., 2012; ZHANG et al., 2017)
IN VITRO PROTEIN DIGESTIBILITY - CORRECTED AMINO ACID SCORE IVPDCAAS	Rapid and low-cost assay; Does not rely on animal experimentation; Replacement for currently recommended in vivo rats bioassays;	$AAS = \frac{\text{Content of first limiting amino acid in a test protein (mg/g)}}{\text{Content of corresponding amino acid in a reference protein (mg/g)}}$ $IVPDCAAS = IVPD\ (\%) \times AAS$	(NOSWORTHY et al., 2017b, 2017a, 2018a, 2018b; NOSWORTHY; HOUSE, 2017)
In vivo			
PROTEIN EFFICIENCY RATIO PER	Ratio of the rat weight gain and the amount of protein consumed; First method adopted; Overestimates the requirements for humans and underestimates the quality of some proteins;	$PER = \frac{\text{Body weight gain in mass (g)}}{\text{Protein intake (g)}}$	(GIAMI, 2004; ALETOR, 2012; BOYE; WIJESINHA-BETTONI; BURLINGAME, 2012; GILANI, 2012; SCHAAFSMA, 2012; HAN; CHEE; CHO, 2015; CODA et al., 2017)
NET PROTEIN RATIO (or RETENTION) NPR	Overcomes the major weakness in the PER assay by adding the weight loss of rats fed a non-protein diet; Underestimates the protein quality, since rats require higher amounts of amino acids than humans;	$NPR = \frac{\text{Weight gain of test rat - Weight loss of protein-free diet test rat}}{\text{Protein consumed by rat}}$	(GIAMI, 2004; SOUSA et al., 2011; ALETOR, 2012; BOYE; WIJESINHA-BETTONI; BURLINGAME, 2012; GILANI, 2012; HAN; CHEE; CHO, 2015)

Table 2 (continue) – Methods frequently used for determination of in vitro protein digestibility and in vivo protein quality (SÁ; MORENO; CARCIOFI, 2019).

Protein quality evaluation methods	Observations	Calculation	References
In vivo			
NET PROTEIN UTILIZATION NPU	Proportion of nitrogen intake (ingested protein) that is retained; Measure of overall protein utilization;	$NPU\ (\%) = \frac{I - (F - M) - (U - E)}{I} . 100$ Where: I = Nitrogen intake in the test group; F = nitrogen excreted in the faeces; U = nitrogen excreted in the urine; M = endogenous faecal nitrogen excreted by protein-free group (basal diet); E = endogenous urinary nitrogen excreted by protein-free group (basal diet);	(SAHARAN; KHETARPAUL, 1994; CHEW; CASEY; JOHNSON, 2003; MONTOYA et al., 2008; ALETOR, 2012; BOYE; WIJESINHA-BETTONI; BURLINGAME, 2012; GILANI, 2012; SUN et al., 2012; AGUILAR et al., 2015; HAN; CHEE; CHO, 2015)
TRUE DIGESTIBILITY TD	Represents the portion of diet nitrogen that is available for maintenance and growth functions;	$TD\ (\%) = \frac{I - F - Fk}{I} . 100$ Where: I = Protein intake of rats fed test diet; F = Protein excreted in faeces of rats fed test diet; Fk = Protein excreted in faeces of rats fed protein-free diet;	(SAHARAN; KHETARPAUL, 1994; CHEW; CASEY; JOHNSON, 2003; GIAMI, 2004; HUGHES et al., 2011; ALETOR, 2012; BOYE; WIJESINHA-BETTONI; BURLINGAME, 2012; HUSSAIN et al., 2012; AGUILAR et al., 2015; HAN; CHEE; CHO, 2015)
BIOLOGICAL VALUE BV	Proportion of the absorbed nitrogen retained for maintenance and growth, taking into consideration the metabolic nitrogen loss;	$BV\ (\%) = \frac{NPU}{TD} . 100$	(SAHARAN; KHETARPAUL, 1994; ALETOR, 2012; BOYE; WIJESINHA-BETTONI; BURLINGAME, 2012; GILANI, 2012; HUSSAIN et al., 2012; SCHAAFSMA, 2012; AGUILAR et al., 2015; HAN; CHEE; CHO, 2015; CODA et al., 2017)
PROTEIN DIGESTIBILITY CORRECTED AMINO ACID SCORE PDCAAS	Based on the ratio of the first-limiting essential amino acid in the test protein to the reference; Underestimates the value of high-quality proteins and overestimates the value of low-quality proteins; Chemical scores exceeding 100 % are truncated;	$AAS = \frac{\text{Content of first limiting amino acid in a test protein (mg/g)}}{\text{Content of corresponding amino acid in a reference protein (mg/g)}}$ PDCAAS (%) = Amino acid score (AAS) x True digestibility (TD) x 100	(SARWAR, 1997; GILANI; SEPEHR, 2003; SCHAAFSMA, 2005, 2012; SOUSA et al., 2011; KNISKERN; JOHNSTON, 2011; BOYE; WIJESINHA-BETTONI; BURLINGAME, 2012; SUN et al., 2012; GILANI, 2012; AGUILAR et al., 2015; HAN; CHEE; CHO, 2015; PENCHARZ; ELANGO; WOLFE, 2016)
DIGESTIBLE INDISPENSABLE AMINO ACID SCORE DIAAS	Tests with pigs for an appropriate estimation for humans, avoiding the flaws of the PDCAAS procedure;	DIAAS (%) = Lowest value of digestible indispensable AA reference x 100	(PENCHARZ; ELANGO; WOLFE, 2016; MATHAI; LIU; STEIN, 2017)

Plant protein digestibility and bioavailability may be a limiting aspect to be evaluated aiming to replace traditional high-quality sources in the human diet. They are affected by protein chemical structure, processing steps, and unfavorable compounds presence (so-called antinutritional factors, ANFs). Some examples of these compounds are the proteases inhibitors (trypsin and chymotrypsin), lectins, phytates, fibers, and polyphenols (tannins) (BARTKIENE; JUODEIKIENE; VIDMANTIENE, 2012; KALPANADEVI; MOHAN, 2013).

ANFs have been reported to adversely affect the protein and amino acid digestibility (GILANI; XIAO; COCKELL, 2012; ANAYA et al., 2015; SHI et al., 2017) by reducing its bioavailability and interfering with metabolic processes, provoking deleterious effects on the gastrointestinal tract physiology (BOYE; WIJESINHA-BETTONI; BURLINGAME, 2012; KAMELA et al., 2016; TUŚNIO et al., 2017; ADENEKAN et al., 2018). Although many authors presented the ANFs as detrimental and disadvantageous for the digestibility of proteins, the term “antinutritional factors” is not adequate because these compounds also have other benefits for human health. For example, studies show that increased fiber intake benefits many gastrointestinal disorders, lowers blood pressure and serum cholesterol levels, and may enhance immune function (ANDERSON et al., 2009; LAMBEAU; MCRORIE, 2017).

Several studies suggest that plant polyphenols have biological activities, such as antioxidant, anti-inflammatory, antibacterial, anticancer, anti-diabetic, and reduce the risks of cardiovascular diseases (FANG; BHANDARI, 2010; ANNOR et al., 2017). Furthermore, phytic acid and phytate display a wide range of bioactivities, including antioxidant, anticancer, cardiovascular protective, and inhibition effects for kidney stone formation (AIDER; BARBANA, 2011; RIZZO; BARONI, 2018). However, regarding protein digestibility, these compounds should be removed to enhance the protein quality of a plant source. Many reports showed that several heat processing techniques might be considered to overcome the adverse factors of these compounds (heat-labile), improve the protein digestibility of plant proteins, and, therefore, their utilization by the human body (CODA et al., 2017; TUŚNIO et al., 2017).

2.4 IMPACT OF FOOD PROCESSING ON THE PROTEIN QUALITY

Conventional processing techniques, such as cooking, dehulling, soaking, germination, drying, irradiation, fermentation, and extrusion have been demonstrated as improving the nutritional quality of plant proteins (SIDDHURAJU; MAKKAR; BECKER, 2002; SHIMELIS; RAKSHIT, 2005; BOYE; WIJESINHA-BETTONI; BURLINGAME, 2012;

SUN et al., 2012; TUŚNIO et al., 2017) and eliminating the compounds that may reduce protein digestibility (BHATTY; GILANI; NAGRA, 2000; KAMELA et al., 2016).

Conventional techniques based on thermal processing are well established to reduce or eliminate these compounds and increase the protein digestibility of the plant proteins. Tables 3 and 4 summarize the influence of conventional processing on protein digestibility. More information regarding the effect of conventional food processing on the protein digestibility of different plant protein sources can be found in Sá; Moreno; Carciofi (2019).

Although the use of processing is beneficial in terms of protein quality by inactivating the compounds that lower the protein digestibility of plant proteins, the chemical changes produced by the heating process may also decrease nutritional benefits by degrading some heat-labile micronutrients, like reducing the assimilation of some vitamins and minerals and provoke the generation of some toxic compounds (CANNIATTI-BRAZACA, 2006). Some detrimental effects of thermal processing can occur, such as protein degradation, due to the Maillard reaction, impacting essential amino acids bioavailability. The non-enzymatic browning has been presumed to affect the quality of the protein due to the blockage of amino acids, and the product formed has proteolytic inhibitor activity that reduces the IVPD (SHIMELIS; RAKSHIT, 2005). Carbonyls may react with other amino acids or polymerize into brown melanoidins, adversely impacting lysine availability and protein digestibility. High processing temperatures may also induce cross-linking, protein-protein interactions, and racemization of amino acids (CHIESA; GNANSOUNOU, 2011; PATTO et al., 2015).

Techniques using mild conditions may balance nutritional aspects beyond protein digestibility and feasible processes with reduced environmental impact. Also, emerging processing techniques, such as pulsed electric field, ultrasound, high-pressure, cold plasma, and enzymatic processes, seem promising to increase protein nutritional value and techno-functionalities since they have been reported to affect protein structure and food composition under mild temperatures. These emerging techniques are promising for plant proteins and can overcome the overheating drawbacks of conventional thermal methods.

These novel approaches have already been explored for the successful inactivation of trypsin inhibitor in plant proteins (VAGADIA et al., 2018) in soybeans (TORREZAN; FRAZIER; CRISTIANINI, 2010; VAGADIA; VANGA; RAGHAVAN, 2017; VAGADIA et al., 2018), chickpeas (ALAJAJI; EL-ADAWY, 2006), and beans (JOURDAN; NOREA; BRANDELLI, 2007).

Table 3 – Influence of thermal processing on the protein digestibility of plant proteins.

Source of plant protein	Food processing	Protein quality evaluation method	Results	Reference
Soybean (*Glycine max*)	Irradiation (10 kGy) Autoclaving (123 ºC, 20 min)	IVPD (%)	89.3 81.3	(LEE et al., 2012)
	Peeling and cooking (100 ºC, 30 min)	IVPD (%)	89.8 ± 0.1	(BERNO; GUIMARÃES-LOPES; CANNIATTI-BRAZACA, 2007)
	Defatted flour Defatted flour and irradiation (1 kGy) Defatted flour and irradiation (5 kGy) Defatted flour and irradiation (10 kGy)	IVPD (%)	79.8 81.2 82.3 84.2	(SIDDHURAJU; MAKKAR; BECKER, 2002)
Bean (*Phaseolus vulgaris* L.)	Raw Autoclaving (121 ºC, 10 min) Raw after 6 months storage Autoclaving after 6 months storage	IVPD (%)	92.8 92.3 95.3 97.9	(DELFINO; CANNIATTI-BRAZACA, 2010)
	Microwave (800 W, 2450 MHz, 1 min) Microwave (800 W, 2450 MHz, 3 min)	IVPD (%)	81.8 85.8	(SHIMELIS; RAKSHIT, 2005)
	Raw Autoclaving (121 ºC, 10 min) Autoclaving and irradiation (2 kGy) Autoclaving and irradiation (6 kGy) Autoclaving and irradiation (10 kGy)	IVPD (%)	84.0 ± 0.3 84.2 ± 0.3 82.2 ± 0.1 84.4 ± 0.5 82.3 ± 0.8	(MECHI; CANIATTI-BRAZACA; ARTHUR, 2005)
	Raw Germination (25 ºC, 72 h)	IVPD (%)	68.1 ± 0.4 78.0 ± 0.3	(ALONSO; AGUIRRE; MARZO, 2000)
	Autoclaving (9 psi, 112 ºC, 30 min)	PER (ratio) NPR	1.9 ± 0.3 3.3 ± 0.4	(YAÑEZ et al., 1995)
	Autoclaving (121 ºC, 15 min)	TD (%)	68.0	(VAN DER POEL, 1990)

Table 3 (continue) – Influence of thermal processing on the protein digestibility of plant proteins.

Source of plant protein	Food processing	Protein quality evaluation method	Results	Reference
Pea (*Pisum sativum* L.)	Raw	IVPD (%)	80.1	(BOYE; WIJESINHA-BETTONI; BURLINGAME, 2012)
		PDCAAS (%)	46	
	Autoclaving (15 psi, 121 ºC, 20 min)	IVPD (%)	88.3	
		PDCAAS (%)	67	
	Microwave (1200 W, 15 min)	IVPD (%)	90.9	
		PDCAAS (%)	92	
	Raw	IVPD (%)	83.5	(PARK; KIM; BAIK, 2010)
	Cooking (98 ºC, 30 min)		86.8	
	Raw	IVPD (%)	73.5 ± 1.3	(HABIBA, 2002)
	Cooking (100 ºC, 40 min)		78.3 ± 1.2	
	Autoclaving (121 ºC, 15 min)		78.3 ± 1.4	
	Microwave (2450 MHz, 12 min)		75.5 ± 1.2	
	Autoclaving (15 psi, 10 min)	IVPD (%)	86.3 ± 0.1	(BISHNOI; KHETARPAUL, 1994)
	Germination (48 h)		82.7 ± 0.1	
	Uncooked flour	PER (ratio)	2.3 ± 0.2	(SAHARAN; KHETARPAUL, 1994)
		TD (%)	66.7 ± 2.2	
		BV (%)	62.9 ± 2.8	
		NPU (%)	42.1 ± 3.0	
		NPR	50.0 ± 1.4	
	Autoclaved (15 psi, 15 min) flour	PER (ratio)	2.5 ± 0.2	
		TD (%)	70.5 ± 1.7	
		BV (%)	67.2 ± 3.1	
		NPU (%)	47.4 ± 3.1	
		NPR	51.2 ± 2.0	
Finger millet (*Eleusine coracana*)	Raw	IVPD (%)	79.0	(ANNOR et al., 2017)
	Cooking		84.7-86.3	
	Germination (30 ºC, 48h)		92.0	

Table 3 (continue) – Influence of thermal processing on the protein digestibility of plant proteins.

Source of plant protein	Food processing	Protein quality evaluation method	Results	Reference
Sweet potato (*Ipomoea batatas* L.)	Raw	IVPD (%)	52.8 ± 0.7	(SUN et al., 2012)
	Cooking (100 ºC, 60 min)		85.7 ± 1.4	
	Microwave (700 W, 3 min)		94.1 ± 1.8	
	Drying (130 ºC, 60 min)		54.7 ± 0.4	
	Autoclaving (127 ºC, 20 min)		99.2 ± 0.1	
	Autoclaving (127 ºC, 20 min)	NPU (%)	92.0 ± 1.0	(SUN et al., 2012)
		TD (%)	95.1 ± 3.1	
		PDCAAS (%)	70.0 ± 0.1	
Chickpea (*Cicer arietinum*)	Raw	PER (ratio)	1.5 ± 0.1	(BHATTY; GILANI; NAGRA, 2000)
		TD (%)	64.6 ± 0.4	
		NPU (%)	36.7 ± 1.1	
	Cooking (100 ºC, 40 min)	PER (ratio)	0.8 ± 0.1	
		TD (%)	77.9 ± 0.7	
		NPU (%)	38.4 ± 0.4	
	Raw	IVPD (%)	71.8 ± 1.0	(CLEMENTE et al., 1998)
	Autoclaving (120 ºC, 50 min)		83.5 ± 0.1	
Fababean (*Vicia faba* L.)	Raw	IVPD (%)	70.8 ± 0.2	(ALONSO; AGUIRRE; MARZO, 2000)
	Germination (25 ºC, 72 h)		78.1 ± 0.2	
	Raw	IVPD (%)	64.6 ± 1.2	(KHALIL; MANSOUR, 1995)
		PER (ratio)	2.4	
	Cooking (45 min)	IVPD (%)	71.2 ± 1.2	
		PER (ratio)	2.7	
	Autoclaving (121 ºC, 30 min)	IVPD (%)	73.7 ± 1.4	
		PER (ratio)	2.6	
	Germination (25ºC, 72h)	IVPD (%)	72.2 ± 1.3	
		PER (ratio)	2.6	

Table 3 (continue) – Influence of thermal processing on the protein digestibility of plant proteins.

Source of plant protein	Food processing	Protein quality evaluation method	Results	Reference
Flaxseed (*Linus usitatissimum*)	Extrusion Experimental design varying screw speed, moisture, temperature and feed rate	IVPD (%)	73.1 - 77.0	(WU et al., 2015)
	Extrusion (95 – 100 ºC)	NPU (%) TD (%) BV (%) NPR	58.4 ± 6.5 73.0 80.0 ± 8.7 3.2 ± 0.3	(GIACOMINO et al., 2013)
	Extrusion Experimental design varying screw speed, moisture, temperature and feed rate	IVPD (%)	69.5 - 77.4	(WANG et al., 2008)
Canola (*Brassica sp.*)	Raw Extrusion (110 ºC)	IVPD (%)	79.5 78.1-81.3	(ZHANG et al., 2017)
Red sorghum (*Sorghum spp*)	Raw Extrusion (182 ºC, 14 % moisture)	IVPD (%)	53.2 ± 2.0 70.0 ± 0.2	(LLOPART et al., 2014)
Corn (*Zea mays*)	Extruded flour (79.4 ºC)	IVPD (%)	80.9	(BOYE; WIJESINHA-BETTONI; BURLINGAME, 2012)
Soybean (*Glycine max*)	Extrusion	IVPD (%)	88.8 ± 0.7	(BERNO; GUIMARÃES-LOPES; CANNIATTI-BRAZACA, 2007)
Fababean (*Vicia faba* L.)	Extrusion (156 ºC, 25 % moisture)	IVPD (%)	87.4 ± 0.2	(ALONSO; AGUIRRE; MARZO, 2000)
Bean (*Phaseolus vulgaris*)	Extrusion (156 ºC, 25 % moisture)	IVPD (%)	83.0 ± 0.3	(ALONSO; AGUIRRE; MARZO, 2000)
	Extrusion (150 ºC, 16 s)	TD (%)	79.0	(VAN DER POEL, 1990)

Table 4 – Influence of fermentation on the protein digestibility of plant proteins.

Source of plant protein	Food processing	Protein quality evaluation method	Results	Reference
Bean (*Phaseolus vulgaris* L.)	Unfermented bean flour Fermented with *Pleurotus ostreatus* (70 ºC)	IVPD (%)	40.0 ± 1.7 48.1 ± 0.8	(ESPINOSA-PÁEZ et al., 2017)
	Unfermented flour Fermented with *Bacillus sp.* protease flour (28 ºC, 5 h)	IVPD (%)	54.4 81.6	(DIAS et al., 2010)
Finger millet (*Eleusine coracana*)	Fermented	IVPD (%)	71.2-83.7	(ANNOR et al., 2017)
Oat (*Avena sativa*)	Unfermented oat flour Fermentation with *Pleurotus ostreatus* (70 ºC)	IVPD (%)	63.3 ± 1.7 70.0 ± 0.3	(ESPINOSA-PÁEZ et al., 2017)
Corn (*Zea mays*) and Soybean (*Glycine max*)	Unfermented meal Fermentation with *B. subtilis* and *E. faecium* (37 ºC, 24 h)	IVPD (%)	78.4 ± 2.0 86.3 ± 2.2	(SHI et al., 2017)
Soybean (*Glycine max*)	Unfermented flour Fermented with *Pediococcus acidilactici* flour (30 ºC, 72 h)	IVPD (%)	75.3 ± 1.2 88.7 ± 0.9	(BARTKIENE; JUODEIKIENE; VIDMANTIENE, 2012)
	Unfermented Fermentation with *Bacillus natto* (25 ºC, 48 h)	IVPD (%)	83.0 90.0	(OJOKOH; YIMIN, 2011)
Kariya (*Hildergardia barteri*)	Raw, unfermented flour Raw, fermented flour (30 ºC, 96 h) Cooked (100 ºC), fermented flour (30 ºC, 96 h)	IVPD (%)	63.7 82.1 85.5	(FAWALE et al., 2017)
Cowpea (*Vigna unguiculata* L.)	Fermentation with *Saccharomyces cerevisiae* (25 ºC, 24 h)	IVPD (%) PDCAAS (%)	84.3 81	(BOYE; WIJESINHA-BETTONI; BURLINGAME, 2012)

However, the literature is scarce in studies correlating these technologies and their effects on the protein digestibility and the amino acid composition of plant proteins. Also, information about the required energy and costs is scarce, and these approaches were mainly performed at a laboratory scale; therefore, the large-scale feasibility still needs further studies (CHEMAT; HUMA; KHAN, 2011; CONTRERAS et al., 2019). More information about the utilization of the emerging approaches for protein quality is presented in Sá et al. (2022).

2.5 FUNCTIONAL PROPERTIES OF PLANT PROTEINS

Protein functionality has critical importance in defining the applicability of plant proteins flours, concentrates, and isolates, which affect the physicochemical characteristics of food products (texture, appearance, stability, cohesion-adhesion, elasticity, and viscosity). Intrinsic and extrinsic factors (e.g., protein structure, amino acid composition, hydrophobicity, medium pH, salts, temperature, pressure, and ionic strength) can influence the functional properties of protein-containing foods (KYRIAKOPOULOU; DEKKERS; VAN DER GOOT, 2019; STONE et al., 2019; GENÇDAĞ; GÖRGÜÇ; YILMAZ, 2020). Protein extraction and processing may change those functional properties; thus, studying the process parameters is essential to understand the impact on food products' functional and physicochemical properties.

Studies evaluated the functional properties of plant proteins, such as soybean, chickpea, kidney bean (BYANJU et al., 2020), pea (XIONG et al., 2018; KYRIAKOPOULOU; DEKKERS; VAN DER GOOT, 2019), lentils (SAMARANAYAKA, 2017), quinoa (RUIZ, 2016), cashew nut (Liu et al. 2018), sorghum (BERNARDO et al., 2019), avocado (WANG et al., 2019), and mustard (CHAKRABORTY; BHATTACHARYYA; GHOSH, 2021).

Few studies investigated the functional properties of edible oil processing by-products regarding solubility from rapeseed/canola (CAMPBELL; REMPEL; WANASUNDARA, 2016; ZHANG et al., 2017). The utilization of plant proteins is limited due to their extremely low solubility at neutral pH, except for the soybean, pea, and canola (CONTRERAS et al., 2019). Other studies also evaluated some plant proteins' foaming capacity and stability, like soybean, pea, chickpea, lupin, and rapeseed (BARAC et al., 2015; TONTUL et al., 2018). These sources have excellent foaming properties, comparable to egg protein, mostly due to high solubility, high surface hydrophobicity, low molecular weight, and net charge (SUN-WATERHOUSE; ZHAO; WATERHOUSE, 2014).

Some plant proteins have highlighted emulsifying properties, like the bell pepper, which formed stabilized emulsions with small oil droplet sizes (Li et al. 2018); peas (BARAC et al., 2015); chickpeas, with high emulsion activity index (EAI) at pH 10 (TONTUL et al., 2018); soybean, with a high emulsifying capability and emulsion stabilization against creaming during storage (CHEN et al., 2011); and rapeseed, with higher emulsifying stabilities than soybean products (AIDER; BARBANA, 2011). Furthermore, few studies evaluated plant proteins' water- and oil-holding capacities (WHC and OHC). Li et al. (2018) studied bell peppers and suggested this source to food products requiring high WHC. He et al. (2014) evaluated the OHC of peanut protein isolates and indicated a remarkably higher value than commercial soybean protein isolates. Although few studies evaluated the gelling properties of plant proteins, there are results about rapeseed products (flours, concentrates, and isolates) reporting poor gelation properties (TAN et al., 2011). However, soybean protein isolates have been the reference material as gelling agents in several semi-solid food products, mainly for meat analogs (BESSADA; BARREIRA; OLIVEIRA, 2019).

Therefore, few studies are available reporting the solubility, emulsifying, foaming, water- and oil-holding capacities, and gelling properties for plant proteins. Potentially, plant-based proteins may be used by the food industry in formulations for protein supplements, meat analogs, beverages, snacks, desserts, bakery, whipped creams, soups, sauces, and salad dressings (KYRIAKOPOULOU; DEKKERS; VAN DER GOOT, 2019). From here, one can consider that exploring plant-based proteins aiming to develop technological alternatives for food formulation is an open field, including evaluating the required processing technologies for extraction and modulating the techno-functionalities. More information about the improvement of functional properties of proteins is presented in Appendix A.

2.6 FINAL CONSIDERATIONS REGARDING THE STATE OF THE ART

There is a constant requirement for protein quality and availability worldwide, covering the food security obligation. Plant protein digestibility and bioavailability are critical aspects of meeting human nutritional needs in a scenario of the world's population increasing and constrained environmental resources, especially when looking for animal-based protein substitution. How to accurately determine and improve the protein quality of a plant source remains a scientific and technological challenge that should be addressed shortly. A developed solution, coupling the plant protein source and a processing technique, needs to fit the

environmental, economic and health requirements, and the consumers' sensory and cultural aspects, including and not limited to tradition, religion, and animal welfare.

Based on the literature review exposed in this chapter, the agro-industrial by-products are a golden opportunity for human nutrition that may be used as food supplements. However, insufficient studies investigate the protein quality of alternatives for plant protein sources in terms of protein digestibility, antinutritional factors concentration, and amino acid profile. Although the literature is scarce in studies correlating emerging technologies and nutritional value of plant proteins from industrial residues, a key question is whether protein from agri-food by-products can be extracted efficiently and cost-effectively. Thus, this field needs to be explored, and efforts are needed to enhance the nutritional quality of plant protein sources. Also, the interest in this field is increasing due to the growing demand for clean technologies, allied to the production of sustainable protein sources and food security.

Furthermore, this work's field meets some of the Sustainable Development Goals (SDGs) of the ONU 2030 Agenda for providing alternative and nutritious protein sources, contributing to the diversification of the human diet (Goal 2: "Zero hunger, food security, and sustainable agriculture" and Goal 3: "Good health and well-being"); for using agro-industrial wastes and adding value to renewable raw materials (Goal 12: "Responsible consumption and production"); and for reducing animal-based protein intake due to its negative environmental impacts (Goal 13: "Climate action"). Additionally, the 2021 United Nations Climate Change Conference – known as COP26 – puts the world on a path to aggressively slow Earth's warming and cut greenhouse gas emissions. Its goals align directly with taking direct action to promote sustainable alternatives to animal agriculture.

Using agro-industrial residues in plant-based products and formulations could be a sustainable choice for minimizing negative environmental impacts, helping solve food security problems and the high demand for protein sources by increased population growth. Therefore, this thesis contributes to finding solutions to a growing world population challenge while meeting the protein requirements, offering new nutritious protein ingredients from alternative sources for food formulations, and meeting the functional properties in developing new products. Finally, the main innovation of this thesis is to interconnect all these topics, using sustainable sources from agro-industrial residues to produce high-quality proteins, enabling the utilization in the nutrition and food area, and expanding the range of nutritious ingredients available to human consumption.

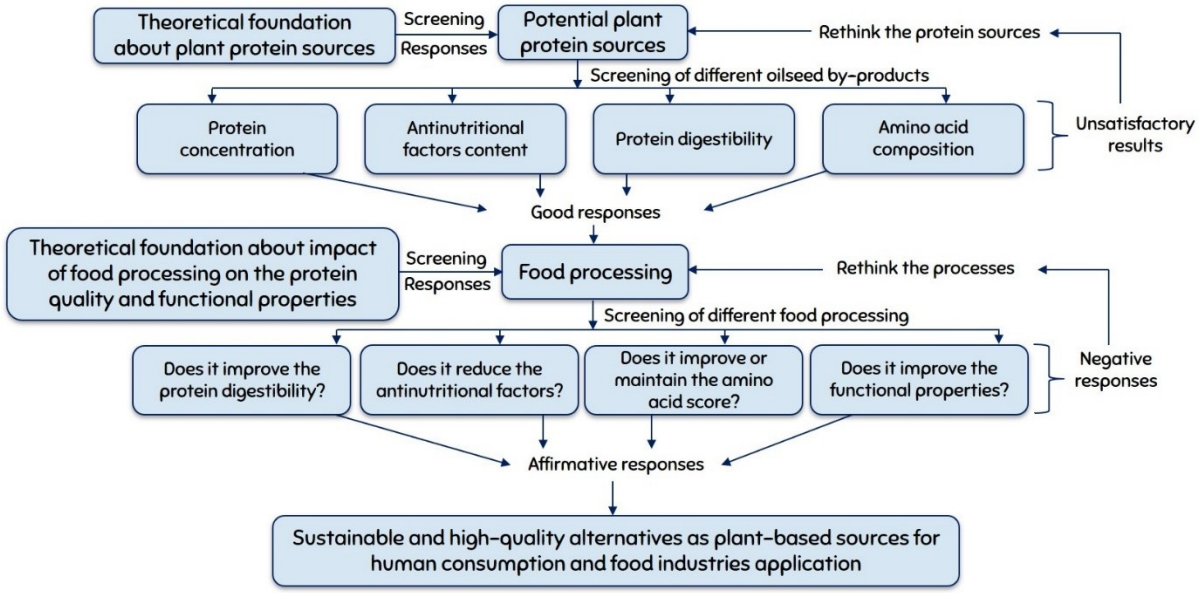
CHAPTER III

3 THESIS STRUCTURE

The production of alternative protein sources includes several challenges that must be overcome to ensure a successful protein extraction process aiming to produce high-quality foods. Most of these challenges are addressed at the choice of the protein source and the optimization of process parameters. Thus, proper screening of potential nutritious protein sources is needed to find excellent ones to carry out the select food processing.

This thesis was structured to overcome these challenges by selecting potential plant protein sources from agro-industrial residues and choosing the best food processing parameters for protein quality improvement. Figure 1 shows the working plan used to explore alternatives for plant proteins and the food processing evaluations in this thesis. Furthermore, after choosing the plant protein and optimizing the process, a study around the viability is crucial to ensure that the scale-up is possible. However, economic and industrial viability is not addressed in this work.

Figure 1 – Working plan for the potential plant protein production in this thesis.



3.1 POTENTIAL PLANT PROTEIN SOURCES

This thesis started with the screening of different oilseed meals from edible oil extraction industrial processing residues. The samples were Pumpkin seed, flaxseed, chia seed, sesame seed, and grapeseed meals, and they were tested for their nutritional composition and protein quality. These results for the nutritional value of the oilseed meals are exposed in Chapter 4.

3.2 FOOD PROCESSING EVALUATION

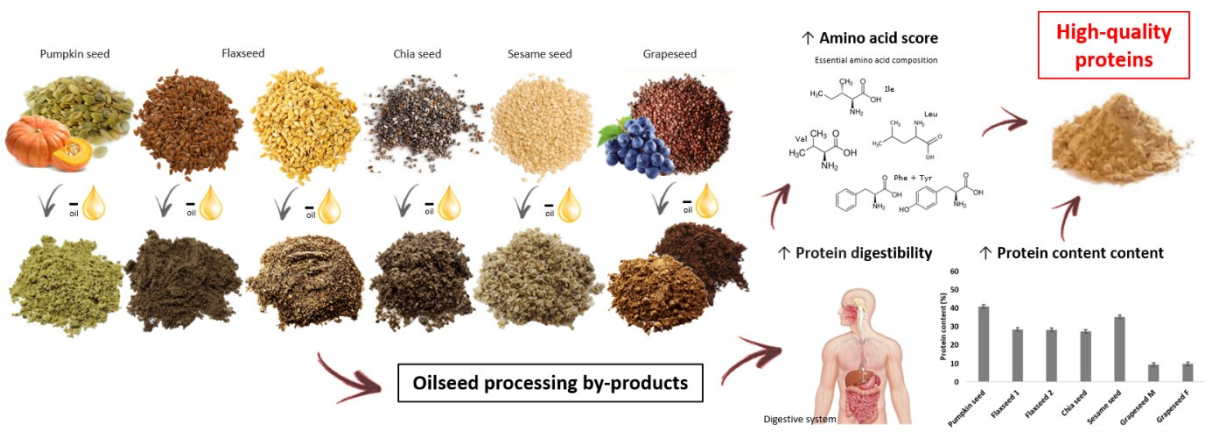
After screening and selecting the protein sources to be processed, different food processing was applied as the second step of this thesis. Cooking, microwave, and ultrasound were chosen to verify the influence of processing on the protein quality of the oilseed meals. The results correlated to protein digestibility, amino acid composition and score, antinutritional factors, and functional properties. These results for the select raw and processed oilseed by-products are exposed in Chapter 5.

CHAPTER IV

4 SCREENING OF OILSEED BY-PRODUCTS AS PROTEIN SOURCES

The utilization of agro-industrial by-products is a feasible alternative to reduce waste disposal and increase limited sources of non-animal proteins. Currently, scarce information about the nutritional quality and protein digestibility of pumpkin seed, flaxseed, chia seed, sesame seed, and grapeseed meals is available in the literature. The aim of this chapter is the determination of the chemical composition, the presence of antinutritional factors (ANFs), the in vitro protein digestibility (IVPD), the amino acid (AA) profile, and the amino acid score (AAS) of these meals. The possibility of obtaining high nutritional valued proteins from these residues is a hypothesis to be validated in this thesis. The results of this chapter are part of the research article D, entitled "Oilseed by-products as plant-based protein sources: Amino acid profile and digestibility", published in the ***Future Foods*** journal (SÁ et al., 2021). The article's graphical abstract is presented in Figure 2.

Figure 2 – Article's graphical abstract (SÁ et al., 2021).



4.1 MATERIALS AND METHODS

4.1.1 Chemicals

The chemicals used in this study are n-hexane (99% P.A., Neon®), ethyl ether (99.8%, Anidrol®), sulfuric acid (P.A., Anidrol®), acetic acid (P.A., Neon®), boric acid (P.A., Neon®), hydrochloric acid (P.A., Neon®), thioglycolic acid (P.S., Neon®), ethanol (99.5%, Anidrol®),

methanol (P.A., Neon®), 2,2′-bipyridyl (purity 99%, Sigma, St. Louis, MO, USA), BAPNA (purity ≥ 98%, Sigma, St. Louis, MO, USA), selenium dioxide (purity > 99%, Sigma, St. Louis, MO, USA), dimethyl sulfoxide (P.A., Neon®), vanillin (Neon®), catechin hydrate (purity ≥ 96%, Sigma, St. Louis, MO, USA), copper sulfate II pentahydrate (P.A., Neon®), sodium hydroxide (P.A., Neon®), ammonium iron(III) sulfate dodecahydrate (purity 99%, Sigma, St. Louis, MO, USA), and sodium salt hydrate of phytic acid (≥ 90% phosphorus, Sigma, St. Louis, MO, USA).

4.1.2 Sample collection

Pumpkin seed (*Cucurbita moschata*) protein meal (PSM) and brown flaxseed (*Linum usitatissimum*) meal (FM1) were kindly provided by Vital Âtman Ltda., São Paulo, Brazil. Flaxseed meal (FM2) was donated by Cisbra Ltda., Rio Grande do Sul, Brazil. Chia seed (*Salvia hispanica*) meal (CSM) was kindly provided by Agropecuaria Produza S.A., Paraguay. Sesame seed (*Sesamum indicum* L.) meal (SSM) was donated by Sésamo Real Ind. Com. Prods. Alims. Ltda., São Paulo, Brazil. Grapeseed (*Vitis labrusca*) meal (GSM) and flour (GSF) were kindly provided by Econatura Produtos Ecológicos e Naturais Ltda., Rio Grande do Sul, Brazil. The oilseed industries cited above employ cold-pressing extraction to obtain oil from the seeds, without organic solvents. The samples were ground and stored at -18 ºC for further analysis.

4.1.3 Analytical methods

4.1.3.1 Proximate composition

The proximate analysis of the raw oilseed by-products was carried out using official AOAC procedures (2012): moisture gravimetrically at 105 ºC for 24 h (method 925.09); ash by calcination using a muffle furnace at 550 ºC (923.03); lipid gravimetrically after n-hexane extraction (920.39); nitrogen by standard Kjeldahl method (954.01); and crude fiber (962.09). All determinations were performed in triplicates. Protein composition was calculated as nitrogen value multiplied by 6.25 as the conversion factor (AOAC INTERNATIONAL, 2012), and total carbohydrate content on a dry basis was estimated by calculating the percentile difference to crude proteins, lipids, ashes, and fibers. The Atwater conversion factors of 9 kcal/g

(for lipids) and 4 kcal/g (for proteins and carbohydrates) (FAO, 2003) were used to estimate the energy value of the samples.

4.1.3.2 Antinutritional factors

4.1.3.2.1 Trypsin inhibitors

The determination of trypsin inhibition activity was performed according to Kakade et al. (1974). The trypsin assay contained trypsin from the bovine pancreas (salt-free lyophilized powder, ≥ 10.000 BAEE units/mg of protein, product no. T1426, Sigma, Chemical, St. Louis, MO, USA) and BAPNA reagent (Nα-Benzoyl-DL-arginine 4-nitroanilide hydrochloride, purity ≥ 98%, product no. B4875, Sigma, Chemical, St. Louis, MO, USA) as substrates. One gram of finely ground sample (80 mesh) was extracted with 50 mL of NaOH 0.01 M for 3 h at room temperature. Supernatant aliquots of 1 mL were pipet into tubes, and 1 mL of distilled water was added. Distilled water (2 mL) was used as a reagent blank. Extracts were incubated with 2 mL of trypsin solution (0.02 mg/mL in 0.001 M HCl) and 5 mL of BAPNA reagent (0.4 mg/mL in Tris-buffer pH 8.2, containing $CaCl_2$) in a water bath at 37 ºC. After 10 min, 1 mL of 30% (v/v) acetic acid was added to terminate the reaction. Trypsin inhibitor activity (TIA) was spectrophotometrically determined at 410 nm (UV/VIS spectrophotometer, Hitachi U-1900) against a reagent blank. The trypsin inhibition activity (TIA) was expressed as the trypsin inhibition unit (TIU) per milligram of the sample.

4.1.3.2.2 Tannins

The tannin content was estimated by the colorimetric method of vanillin-HCl, as described by Burns (1971). The tannins were extracted for 24 h at room temperature, which 1 g of finely ground sample (80 mesh) was mixed with 50 mL of methanol. Supernatant aliquots of 1 mL were pipet into tubes, and 5 mL of vanillin-HCl reagent was added. Then, the colored solution was measured spectrophotometrically at 500 nm (UV/VIS spectrophotometer, Hitachi U-1900). Catechin ((+)-Catechin hydrate, purity ≥ 96%, product no. 22110, Sigma, Chemical, St. Louis, MO, USA) was used as the reference standard, and the tannin concentration was expressed in mg catechin per gram of sample.

4.1.3.2.3 Phytic acid

The phytic acid content was estimated by the methodology of Haug and Lantzsch (1983). The samples were extracted for 24 h at room temperature, which 1 g of finely ground sample (80 mesh) was mixed with 50 mL of 0.2 N HCl. Supernatant aliquots of 0.5 mL were pipet into tubes, and 1 mL of ferric solution (ammonium iron (III) sulfate dodecahydrate, purity 99%, product no. 221260, Sigma, Chemical, St. Louis, MO, USA) was added, and tubes were put in a boiling water bath (100 ºC) for 30 min. At room temperature, 1.5 mL of the 2,2'-bipyridine (purity ≥ 99%, product no. D216305, Sigma, Chemical, St. Louis, MO, USA) solution was added. Then, the colored solution was measured spectrophotometrically at 519 nm (UV/VIS spectrophotometer, Hitachi U-1900). Sodium salt hydrate of phytic acid (≥ 90% phosphorus, product no. 68388, Sigma, Chemical, St. Louis, MO, USA) was used as a phytate reference solution for the standard calibration curve. The phytic acid was estimated as µg per gram of sample.

4.1.3.3 In vitro protein digestibility (IVPD)

The Hsu et al. (1977) method with minor modifications (TINUS et al., 2012) was used to determine the IVPD of oilseed by-products. The protein suspension (6.25 mg/mL of distilled water) was adjusted to pH 8.0 with 0.1 N NaOH or 0.1 M HCl while stirring at 37 °C. An enzyme mix containing 1.6 mg of trypsin (porcine pancreatic trypsin type IX-S, 13.000-20.000 BAEE units/mg protein, product no. T0303, Sigma, Chemical, St. Louis, MO, USA), 1.3 mg of peptidase (porcine gastric mucosa pepsin, 3.200 – 4.500 units/mg protein, product no. P6887, Sigma, Chemical, St. Louis, MO, USA), and 3.1 mg of α-chymotrypsin (bovine pancreatic chymotrypsin type II, ≥ 40 units/mg protein, product no. C4129, Sigma, Chemical, St. Louis, MO, USA) per mL was maintained in an ice-bath and adjusted to pH 8.0. The enzymatic solution was added to the protein solution at a 1:10 v/v ratio and stirred at 37 ºC. A rapid decrease in pH value occurred due to the amino acid carboxyl groups releasing from the protein chain by the proteolytic enzymes. The pH mixture was measured after 10 min using a portable pH meter (model testo 205, Testo Instrument Co.). IVPD as a percentage of digestible protein was estimated according to pH variation after 10 min (ΔpH_{10min}), as shown in Equation 1.

$$IVPD\ (\%) = 65.66 + 18.10 \times \Delta pH_{10min} \quad (1)$$

4.1.3.4 Amino acid composition

The determination of total amino acids of the raw material was performed by reverse phase column (C18 from Phenomenex) chromatography in a high-performance liquid chromatograph (HPLC, SHIMADZU®), according to the method described in Hagen et al. (1989). The release of individual amino acids occurs in acid hydrolysis at 110 ºC for 22 h, using 6 M of hydrochloric acid and phenol solutions. After the hydrolysis, α-aminobutyric acid (Sigma-Aldrich®, St. Louis, MO, USA) is added as an internal standard. The identification of the amino acids was performed by comparison with an external standard (Pierce, PN 20088). The internal standard α-aminobutyric acid was used for the quantification of total amino acids, according to White et al. (1986) method.

4.1.3.5 Amino acid score and in vitro protein digestibility-correct amino acid score (IVPDCAAS)

The amino acid composition of the samples was used to estimate the Amino Acid Score (AAS) as [mg of amino acid in 1 g of test protein/mg of amino acid in requirement pattern]×100 (WHO/FAO/UNU EXPERT CONSULTATION, 2007). The lowest AAS calculated reflects the first limiting amino acid in the protein source (NOSWORTHY et al., 2017a), and the IVPDCAAS was calculated as a product of the AAS and IVPD values for each sample evaluated (NOSWORTHY et al., 2018b).

4.1.4 Statistical analysis

The software Statistica® (v.13.5, Statsoft Inc.) was used to perform the experimental data statistical analysis, adopting a confidence level of 95% in all cases. The Tukey's test was used to compare the chemical composition, ANFs, and IVPD of oilseed by-products. Results are expressed as mean ± standard deviation of replicated samples.

4.2 RESULTS AND DISCUSSION

4.2.1 Proximate composition

The chemical composition and energy values of the oilseed by-products are presented in Table 5. In terms of protein content, the dry weight basis results for PSM are higher in comparison for edible whole pumpkin seeds reported in the literature (30.2–36.5%) (EL-ADAWY; TAHA, 2001; ROGERSON, 2017) and fibers (4.4–12.1%) (EL-ADAWY; TAHA, 2001; GARCÍA-AGUILAR et al., 2015).

For FM1 and FM2, fiber and protein content are higher than edible whole flaxseed reported in the literature (4.8% and 20.3%, respectively) (KAJLA; SHARMA; SOOD, 2015). Wu et al. (2012) also studied the composition of flaxseed meal, and the protein content was higher (32.7%) than the results presented here. The CSM and SSM results for protein content are similar to those found in the literature for edible whole chia seeds (OLIVOS-LUGO; VALDIVIA-LÓPEZ; TECANTE, 2010) and sesame seeds. The protein results for GSM and GSF are also similar to other studies (8.2–11.8%) (FANTOZZI, 1981; KAMEL; DAWSON; KAKUDA, 1985). The PSM, FM1, FM2, CSM, and SSM contain high protein content (35–41%). Similar results are reported in the literature for oilseed meals (up to 50%) (SARKER et al., 2015; TERRIEN, 2017). These results are also comparable to other meals, such as watermelon seed (27.6%) (LAKSHMI; KAUL, 2011), rapeseed (32.8%) (JIA et al., 2021), black mustard seed (38.2%), and yellow mustard seed (28.8%) (SARKER et al., 2015). Furthermore, comparing these sources to the traditional plant protein sources in the human diet, soybean, common beans, and peas present protein content of 35.3%, 19.9%, and 21.7%, respectively (TERRIEN, 2017).

Nevertheless, the protein intake in the human diet is predominantly animal-based proteins, such as UHT milk (3.5% of protein in whole product (w.p.) / 27.8% in a dry weight basis (d.b.)) (PESTANA et al., 2015), eggs (6.5% (w.p.) / 10.9% (d.b.)) (MURCIA et al., 1999), meat from chicken breasts (20.9% (w.p.) / 58.6% (d.b.)) (FAKOLADE, 2015), and meat from beef steaks (23.1% (w.p.)/ 84.6% (d.b.)) (WAHRMUND-WYLE; HARRIS; SAVELL, 2000). Therefore, the results presented here for the PSM, FM1, FM2, CSM, and SSM show the potential of these residues as high protein sources in food formulations and human nutrition.

Table 5 – Proximate composition and energy values of oilseed by-products (raw samples).

Samples	Moisture (%)	% Dry weight basis					Energy
		Protein[1]	Lipids	Ash	Crude fiber	Carbohydrate[2]	(kcal/100g)
PSM	8.84 ± 0.02^{f}	40.9 ± 0.1^{d}	14.1 ± 0.1^{c}	4.92 ± 0.01^{c}	27.5 ± 0.5^{c}	12.57	340.87
FM1	10.14 ± 0.06^{g}	28.6 ± 0.7^{a}	11.6 ± 0.1^{b}	5.57 ± 0.03^{d}	11.9 ± 0.6^{b}	42.33	388.12
FM2	8.42 ± 0.01^{e}	28.3 ± 0.8^{a}	13.6 ± 0.6^{bc}	6.08 ± 0.01^{e}	10.3 ± 0.5^{ab}	41.72	402.47
CSM	8.06 ± 0.05^{d}	27.5 ± 0.8^{a}	5.4 ± 0.4^{a}	7.15 ± 0.05^{f}	26.7 ± 0.9^{c}	33.25	291.60
SSM	4.27 ± 0.03^{a}	35.3 ± 0.7^{c}	32.8 ± 0.1^{d}	8.26 ± 0.06^{g}	8.2 ± 0.1^{a}	15.44	498.16
GSM	4.58 ± 0.01^{b}	9.4 ± 0.3^{b}	7.1 ± 0.5^{a}	2.31 ± 0.01^{a}	58.6 ± 0.7^{e}	22.59	191.86
GSF	5.34 ± 0.02^{c}	9.9 ± 0.1^{b}	5.8 ± 0.1^{a}	2.48 ± 0.01^{b}	51.4 ± 0.4^{d}	30.42	213.48

All values are means ± standard deviation.

$^{a–g}$ Different letters in the same column indicate a significant difference ($p < 0.05$ by Tukey's test).

[1] N x 6.25.

[2] The available carbohydrate content was determined by calculating the percentile difference from all the other constituents according to the formula: [100 g dry weight - (g crude protein + g lipids + g ash + g crude fiber)].

PSM: pumpkin seed meal; FM1: brown flaxseed meal; FM2: flaxseed meal; CSM: chia seed meal; SSM: sesame seed meal; GSM: grapeseed meal; GSF: grapeseed meal flour.

Additionally, all the oilseed meals present high amounts of dietary fibers (8–59 g/100 g). In terms of dietary fibers, a high source contains > 6 g/100 g (WHO, 2004); therefore, all by-products evaluated in this work are considered a high dietary fiber source.

In terms of vegetable materials, it is well known that any genotype composition can vary depending on the climate, production site, soil type, cultural practices, and even the process of oil extraction (e.g., the use of high or low temperatures, presence of organic solvents, equipment features, pressing capacity, among others conditions), which could bring significant differences on the composition of these oilseeds. This uncertainty in genotype expression may justify some differences presented in this work compared to the literature. The oilseed meal samples were obtained employing cold-pressing extraction without organic solvents. This extraction technique presents great advantages (e.g., higher quality of the oil extracted); however, it can present lower oil yields than oil extraction using high temperature and organic solvents. The results of lipids (on a dry weight basis) demonstrated that oil residual is still presented in the oilseed meals, where SSM has the higher content (32.8%), and CSM has the lower content (5.4%), which dilutes the concentration of other nutrients in the proximate composition. The elimination of the oil residual factor will increase the concentration of the other constituents. Therefore, the concentrations of protein, ash, and crude fiber on a lipid-free basis were calculated, and the results are presented in Table 6.

Table 6 – Protein, ash, and fiber composition of oilseed by-products in a dry weight and lipid-free basis.

Samples	**% Dry weight and lipid-free basis**		
	Protein	**Ash**	**Crude fiber**
PSM	47.6	5.7	32.0
FM1	32.4	6.3	13.5
FM2	32.8	7.0	11.9
CSM	29.1	7.6	28.2
SSM	52.5	12.3	12.2
GSM	10.1	2.5	63.0
GSF	10.5	2.6	54.4

PSM: pumpkin seed meal; FM1: brown flaxseed meal; FM2: flaxseed meal; CSM: chia seed meal; SSM: sesame seed meal; GSM: grapeseed meal; GSF: grapeseed meal flour.

4.2.2 Antinutritional factors

The presence of trypsin inhibitors, phytates, and tannins in food by-products from plant origin are unfavorable for protein digestion; therefore, they must be removed to increase protein digestibility (SÁ; MORENO; CARCIOFI, 2019). The significance of phytates and tannins lies in the extent of their influence on the bioaccessibility of minerals, and the trypsin inhibitors, as the term itself indicate, inhibit protein absorption on binding with proteases (LAKSHMI; KAUL, 2011). These compounds are naturally synthesized due to plant physiology, at the beginning of seed formation (trypsin inhibitors) or the plant healing process (tannins) and during maturation (phytic acid) (SÁ; MORENO; CARCIOFI, 2019). The characterization of the oilseed by-products in terms of these so-called antinutritional factors, such as trypsin inhibition activity, the tannin, and phytic acid concentration, is shown in Table 7.

The raw residues from the oil extraction industries showed elevated trypsin inhibitor activity (11–39.4 TIU/mg). These results are higher in comparison for rapeseed meal (1.74 TIU/mg) (MANSOUR et al., 1993a) and other oilseeds, such as paprika seed flour (1.96 TIU/mg), watermelon seed flour (1.46 TIU/mg), and pumpkin seed flour (1.39 TIU/mg) (EL-ADAWY; TAHA, 2001). The trypsin inhibitor activities for oilseed by-products are also higher when compared to some traditional sources of plant proteins, such as pea (1.84–2.2 TIU/mg) (FRIAS et al., 2011) and lentil (5.12 TIU/mg) (SAMARANAYAKA, 2017). The results are similar to those found for common beans (18.1 TIU/mg) (NIKMARAM et al., 2017). However, the TIA presented here for oilseed residues are lower than soybean (41.5–96.9 TIU/mg) (LUSAS; RHEE, 1995; SAMARANAYAKA, 2017), *Mucuna pruriens* seeds (78.7 TIU/mg) (SIDDHURAJU; VIJAYAKUMARI; JANARDHANAN, 1996), and karkade seed flour (41 TIU/mg) (ABU-TARBOUSH; AHMED, 1996).

The highest level of tannin was noticed in GSM (282 mg/g), which was already expected due to the grape be a rich source of tannins. The other oilseed by-products did not contain tannins. The results for GSM and GSF were higher in comparison to rapeseed meal (9–15 mg/g) (WANASUNDARA et al., 2017), and other oilseeds, such as pumpkin seed flour (1.7 mg/g), watermelon seed flour (2.4 mg/g), and paprika seed flour (4.8 mg/g) (EL-ADAWY; TAHA, 2001). The concentration of tannin in GSM is also higher when compared to traditional sources of plant proteins, such as pea (2.06 mg/g) (FRIAS et al., 2011) and common bean (0.65 mg/g) (ESPINOSA-PÁEZ et al., 2017).

Table 7 – Antinutritional factors concentration and in vitro protein digestibility of oilseed by-products (raw samples).

Samples	TIA (TIU/mg sample)	Tannins (mg catechin/g sample)	Phytic acid (µg/g sample)	IVPD (%)
PSM	12.7 ± 1.0[b]	n.d.[a]	37.0 ± 0.1[e]	85.4 ± 0.5[b]
FM1	30.8 ± 3.0[a]	n.d.[a]	28.1 ± 0.1[b]	83.3 ± 0.1[ab]
FM2	33.6 ± 4.0[a]	n.d.[a]	27.0 ± 0.8[b]	83.9 ± 0.1[ab]
CSM	11.0 ± 1.0[b]	n.d.[a]	18.7 ± 0.8[c]	81.1 ± 0.8[a]
SSM	39.4 ± 4.0[a]	n.d.[a]	23.0 ± 0.4[d]	81.4 ± 0.2[a]
GSM	29.2 ± 1.0[a]	282 ± 6[c]	n.d.[a]	70 ± 1.0[c]
GSF	36.9 ± 2.0[a]	163 ± 8[b]	n.d.[a]	71 ± 2.0[c]

n.d. = not detected.

All values are means ± standard deviation.

[a–e] Different letters in the same column indicate a significant difference ($p < 0.05$ by Tukey's test).

PSM: pumpkin seed meal; FM1: brown flaxseed meal; FM2: flaxseed meal; CSM: chia seed meal; SSM: sesame seed meal; GSM: grapeseed meal; GSF: grapeseed meal flour.

The highest level of phytic acid was noticed in PSM (0.0037 g/100 g). This result is lower than those found in the studies for pumpkin seed flour (2.37 g/100 g) (EL-ADAWY; TAHA, 2001) and pumpkin seeds (0.299 g/100 g) (GIAMI, 2004). The results presented here, in terms of phytic acid composition, are also lower than other oilseed meals, such as watermelon seed (0.99 g/100 g) (LAKSHMI; KAUL, 2011), and rapeseed (or canola) (3.3 g/100 g) (WANASUNDARA et al., 2017), and traditional sources of plant proteins, such as pea (0.35–1.19 g/100 g) (FRIAS et al., 2011), soybean (1–2 g/100 g) (GILANI; LEE, 2003), chickpea (0.12–1.5 g/100 g) (DADON; ABBO; REIFEN, 2017), common bean (1.59 g/100 g) (ALONSO; AGUIRRE; MARZO, 2000), and rice (0.74 g/100 g) (ALBARRACÍN; JOSÉ GONZÁLEZ; DRAGO, 2015). The low phytic acid content may be a consequence of the oil extraction processing that changes the original chemical composition due to chemical affinity. This result highlights the importance of the oil extraction step on improving protein digestion by reducing this ANF.

Results appointed in this study are different from those into the literature, which could be directly associated with the oil extraction process leading to remove some oilseeds ANFs. However, it does not exclude the intrinsic differences due to climate, soil type, production site, cultural practices, and others. At the present moment, no data in the literature was reported about the composition of antinutritional factors in pumpkin seed, flaxseed, chia seed, sesame seed, and grapeseed meals. The antinutritional factors evaluated in terms of the content of phytic acid, tannins, and trypsin inhibitor activity indicated the PSM, FM1, FM2, CSM, SSM, GSM, and GSF as promising sources of proteins for humans.

4.2.3 In vitro protein digestibility (IVPD)

The IVPD is a useful tool for evaluating the nutritive quality of a food protein, combined with the amino acid composition and bioavailability (SÁ; MORENO; CARCIOFI, 2019). The results of IVPD for all the raw oilseed meals evaluated are shown in Table 7.

The protein digestibility presented significant differences ($p < 0.05$) among the by-product raw samples, where PSM presented the highest IVPD (85%), and the GSM and GSF presented the lowest (70%). The results of IVPD presented here for the PSM were higher compared to another study (71.3%) (VENUSTE et al., 2013). The same occurred to the results of FM1 and FM2, where Wu et al. (2012) found 66% of IVPD. The result for CSM was similar to those found in the literature for chia seeds (77.5%) (LÓPEZ et al., 2018); and results for

GSM and GSF were similar to grapeseeds (58–77%) reported in the literature (FANTOZZI, 1981). The IVPD result for the SSM was also higher than another study (74.1%) (EL-ADAWY, 1995).

All by-products IVPD results presented in this work are similar to the protein digestibility of other kinds of residues, such as black and yellow mustard cakes (80.3% and 77.4%, respectively) (SARKER et al., 2015). These results corroborate the potential of these oilseed by-products to be an alternative protein source for human consumption.

4.2.4 Amino acid composition

The amino acid (AA) composition of the raw samples of oilseed by-products is presented in Table 8. The total AA content shows similarity to those results presented for protein content (Table 5), which corroborates the analysis's veracity. For each amino acid evaluated, the raw samples of oilseed by-products showed a statistical difference between them ($p < 0.05$). However, the total essential amino acids (EAA) and non-essential amino acids (NEAA) showed excellent results for these alternative sources of protein.

According to the Amino Acid Score, shown in Table 9, the oilseed meals have a good profile of EAA, although they presented some deficiency in some amino acids. Nevertheless, following the pattern of essential amino acids (WHO/FAO/UNU EXPERT CONSULTATION, 2007), the CSM met the nutritional requirements entirely. The limiting amino acids of the PSM were sulfur amino acids (first limiting amino acid), threonine, and histidine. The first limiting amino acid for FM1 and SSM was lysine, and for FM2, GSM and GSF were sulfur amino acids. The FM1, FM2, and SSM results were very close to those found for the whole flaxseed seed and the defatted sesame seed, respectively. Also, the CSM results in this study were higher than the chia seed after isolation procedures (SÁ; MORENO; CARCIOFI, 2020). However, at the present moment, very few data in the literature were reported regarding the amino acid composition in pumpkin seed, flaxseed, chia seed, sesame seed, and grapeseed meals.

Concerning the in vitro protein digestibility-correct amino acid score (IVPDCAAS), the results are shown in Table 9 for the raw oilseed by-products. The best result of IVPDCAAS was for the chia seed meal (81.1%), the same for the IVPD, due to this by-product did not show any EAA deficiency; followed by flaxseed meals (75.8% and 67.1%), sesame seed meal (54.5%), pumpkin seed meal (50.4%), and grapeseed meals (37.1% and 27.0%).

Table 8 – Amino acid composition (g/100g of protein) of the raw oilseed by-products.

AA composition (g/100g protein)	Samples						
	PSM	FM1	FM2	CSM	SSM	GSM	GSF
Essential (EAA)							
Histidine (His)	1.48 ± 0.06^{AB}	2.47 ± 0.01^{C}	1.32 ± 0.05^{A}	2.10 ± 0.40^{BC}	2.56 ± 0.01^{C}	1.69 ± 0.04^{AB}	1.71 ± 0.05^{AB}
Isoleucine (Ile)	4.05 ± 0.03^{A}	4.50 ± 0.01^{C}	4.62 ± 0.03^{D}	4.01 ± 0.01^{A}	3.99 ± 0.01^{A}	4.38 ± 0.01^{B}	4.38 ± 0.01^{B}
Leucine (Leu)	6.60 ± 0.03^{D}	5.97 ± 0.01^{B}	6.21 ± 0.01^{C}	6.77 ± 0.05^{A}	6.72 ± 0.04^{A}	7.28 ± 0.01^{E}	7.43 ± 0.02^{F}
Lysine (Lys)	4.66 ± 0.02^{E}	4.11 ± 0.01^{C}	4.27 ± 0.01^{D}	4.87 ± 0.05^{F}	3.00 ± 0.01^{B}	3.67 ± 0.01^{A}	3.61 ± 0.01^{A}
Threonine (Thr)	1.39 ± 0.08^{B}	4.04 ± 0.01^{A}	4.19 ± 0.01^{A}	3.95 ± 0.02^{A}	3.85 ± 0.01^{A}	1.80 ± 0.20^{BC}	2.00 ± 0.20^{C}
Valine (Val)	4.69 ± 0.03^{A}	5.32 ± 0.01^{C}	5.40 ± 0.03^{C}	4.91 ± 0.03^{D}	4.75 ± 0.01^{A}	5.15 ± 0.01^{B}	5.19 ± 0.01^{B}
Total sulfur amino acids (Met + Cys)	1.30 ± 0.05^{A}	3.39 ± 0.02^{B}	1.70 ± 0.10^{A}	3.65 ± 0.08^{BC}	4.50 ± 0.20^{C}	1.20 ± 0.50^{A}	0.84 ± 0.01^{A}
Total aromatic amino acids (Phe + Tyr)	10.09 ± 0.04^{D}	7.20 ± 0.01^{A}	7.56 ± 0.01^{AB}	8.75 ± 0.05^{C}	8.48 ± 0.02^{BC}	7.50 ± 0.02^{AB}	7.00 ± 0.70^{A}
Non-essential (NEAA)							
Alanine (Ala)	3.63 ± 0.05^{C}	4.69 ± 0.01^{A}	4.82 ± 0.01^{A}	5.14 ± 0.02^{D}	4.65 ± 0.02^{A}	4.30 ± 0.10^{B}	4.34 ± 0.09^{B}
Arginine (Arg)	14.00 ± 1.00^{C}	10.00 ± 0.01^{A}	10.46 ± 0.04^{A}	10.99 ± 0.06^{A}	13.46 ± 0.02^{C}	7.85 ± 0.01^{B}	7.82 ± 0.02^{B}
Aspartic acid (Asp)	11.94 ± 0.03^{E}	11.26 ± 0.02^{C}	11.39 ± 0.04^{D}	10.21 ± 0.03^{A}	9.61 ± 0.02^{B}	10.10 ± 0.03^{A}	10.17 ± 0.02^{A}
Glutamic acid (Glu)	20.40 ± 0.10^{A}	21.31 ± 0.01^{C}	21.88 ± 0.03^{D}	19.30 ± 0.10^{B}	20.56 ± 0.04^{A}	25.40 ± 0.05^{E}	25.69 ± 0.04^{F}
Glycine (Gly)	7.30 ± 0.70^{A}	6.52 ± 0.01^{A}	6.57 ± 0.01^{A}	5.23 ± 0.08^{B}	5.25 ± 0.02^{B}	9.39 ± 0.04^{C}	9.60 ± 0.10^{C}
Proline (Pro)	3.65 ± 0.01^{A}	4.06 ± 0.02^{B}	4.36 ± 0.02^{C}	4.23 ± 0.01^{BC}	3.82 ± 0.03^{A}	5.40 ± 0.08^{D}	5.68 ± 0.09^{E}
Serine (Ser)	4.90 ± 0.20^{A}	5.15 ± 0.03^{AB}	5.16 ± 0.01^{AB}	5.92 ± 0.03^{B}	4.82 ± 0.02^{A}	4.89 ± 0.02^{A}	4.60 ± 0.50^{A}
Total EAA (g/100g protein)	34.26 ± 0.07^{C}	37.01 ± 0.01^{B}	35.30 ± 0.10^{D}	39.00 ± 0.10^{E}	37.80 ± 0.20^{B}	32.70 ± 0.30^{A}	32.20 ± 0.60^{A}
Total NEAA (g/100g protein)	65.74 ± 0.07^{E}	62.99 ± 0.01^{A}	64.70 ± 0.10^{D}	61.00 ± 0.10^{C}	62.20 ± 0.20^{A}	67.30 ± 0.30^{B}	67.80 ± 0.60^{B}
Total AA (g/100g sample)	36.10 ± 0.10^{F}	28.93 ± 0.02^{A}	29.09 ± 0.08^{A}	25.90 ± 0.20^{D}	34.40 ± 0.06^{E}	9.12 ± 0.01^{B}	9.67 ± 0.02^{C}

$^{A–F}$ Different letters in the same line indicate a significant difference between the raw samples for each amino acid ($p < 0.05$ by Tukey's test).

CSM: chia seed meal; FM1: brown flaxseed meal; FM2: flaxseed meal; GSM: grapeseed meal; GSF: grapeseed meal flour; PSM: pumpkin seed meal; SSM: sesame seed meal.

Table 9 – Amino Acid Score for adults and IVPDCAAS of the oilseed meals.

Amino acids	Requirement pattern[1] (g/100g protein)	Amino Acid Score (%)[2]						
		PSM	FM1	FM2	CSM	SSM	GSM	GSF
Essential								
Histidine (His)	1.5	99 ± 4^{AB}	165 ± 1^{C}	88 ± 4^{A}	138 ± 26^{BC}	170 ± 1^{C}	113 ± 3^{AB}	114 ± 3^{AB}
Isoleucine (Ile)	3.0	135 ± 1^{A}	150 ± 1^{C}	154 ± 1^{D}	134 ± 1^{A}	133 ± 1^{A}	146 ± 1^{B}	146 ± 1^{B}
Leucine (Leu)	5.9	112 ± 1^{D}	101 ± 1^{B}	105 ± 1^{C}	115 ± 1^{A}	114 ± 1^{A}	123 ± 1^{E}	126 ± 1^{F}
Lysine (Lys)	4.5	104 ± 1^{E}	91 ± 1^{C}	95 ± 1^{D}	108 ± 1^{F}	67 ± 1^{B}	82 ± 1^{A}	80 ± 1^{A}
Threonine (Thr)	2.3	61 ± 4^{B}	176 ± 1^{A}	182 ± 1^{A}	172 ± 1^{A}	167 ± 1^{A}	80 ± 11^{BC}	89 ± 10^{C}
Tryptophan (Trp)	0.6	-	-	-	-	-	-	-
Valine (Val)	3.9	120 ± 1^{A}	137 ± 1^{C}	139 ± 1^{C}	126 ± 1^{D}	122 ± 1^{A}	132 ± 1^{B}	133 ± 1^{B}
Total sulfur amino acids (Met + Cys)	2.2	59 ± 3^{A}	154 ± 1^{B}	80 ± 5^{A}	166 ± 3^{BC}	204 ± 10^{C}	53 ± 24^{A}	38 ± 1^{A}
Total aromatic amino acids (Phe + Tyr)	3.8	266 ± 1^{D}	190 ± 1^{A}	199 ± 1^{AB}	230 ± 1^{C}	223 ± 1^{BC}	197 ± 1^{AB}	184 ± 19^{A}
First limiting amino acid	-	Met + Cys	Lys	Met + Cys	-	Lys	Met + Cys	Met + Cys
IVPDCAAS (%)	-	50.4	75.8	67.1	81.1	54.5	37.1	27.0

[1] WHO/FAO/UNU (2007) Expert Consultation Report for adults.

[2] Amino Acid Score: (mg of amino acid in 1 g of test protein/mg of amino acid in requirement pattern)×100.

[A–F] Different letters in the same line indicate a significant difference between the raw samples for each essential amino acid ($p < 0.05$ by Tukey's test).

IVPDCAAS: In Vitro Protein Digestibility-Corrected Amino Acid Score = AAS × IVPD.

CSM: chia seed meal; FM1: brown flaxseed meal; FM2: flaxseed meal; GSM: grapeseed meal; GSF: grapeseed meal flour; PSM: pumpkin seed meal; SSM: sesame seed meal.

These findings agree with some authors, reporting IVPDCAAS for pea flour and protein concentrate (67.0%) (ÇABUK et al., 2018; KONIECZNY et al., 2020), lentil concentrate (55.9%), and faba bean concentrate (33.9%) (NOSWORTHY; HOUSE, 2017).

4.3 FINAL CONSIDERATIONS OF THE CHAPTER

This study showed high nutritional value proteins from agro-industrial wastes, such as the oilseed meals from edible oil processing industries, as sustainable alternative protein sources. Besides, all by-products evaluated in this chapter are also considered a high source of dietary fibers. Among all the samples, chia seed has the most excellent amino acid profile since it is a full source of essential amino acids. Other oilseed by-products evaluated are also good sources, presenting first limiting amino acid as the lysine (sesame seed and brown flaxseed) or sulfur amino acids (pumpkin seed, grapeseed, and flaxseed).

This chapter presents novelty results in the literature since it is the first evaluation for the concentration of antinutritional factors (e.g., tannins, phytic acid, and trypsin inhibitor activity) of flaxseed, chia seed, pumpkin seed, sesame seed, and grapeseed meals, presenting promising results. The trypsin inhibitor activity in all by-products was similar to the value found in traditional sources of plant proteins (e.g., soybean); tannins presented high content only in grapeseed, as expected; and, worthy of highlighting, the phytic acid concentration was lower than most plant protein sources in the literature.

Furthermore, the protein digestibility ranged from 70 to 85% of IVPD, a relatively high value for a plant protein source. However, further processing interventions can improve these values. Regarding the chemical composition (especially protein content), the antinutritional factors concentration, the in vitro protein digestibility, and the amino acid composition, it was possible to determine a ranking for choosing the best raw source among these screening of potential sources, as presented in Table 10 as follows: chia seed meal > brown flaxseed meal > sesame seed meal > pumpkin seed meal > flaxseed meal > grapeseed meal > grapeseed meal flour. These oilseed meals as by-products from the oil extraction industries are high nutritional value protein sources. They are potentially alternative protein sources for human consumption due to the three key factors: low content of antinutritional factors, valuable content of essential amino acids, and good digestibility, which are comparable to the traditional plant-based protein sources such as soybean, beans, and peas.

Table 10 – Factors for selecting the best protein sources among the screening of oilseed meals.

Samples	High protein content (> 25%)	Low content of ANFs			High IVPD (> 80%)	High content of EAA
		TIA (< 15 TIU/mg)	Tannins (Not detected)	Phytic acid (< 2 mg/g)		≤ 1 Deficiency
PSM	✔	✔	✔	✔	✔	X
FM1	✔	X	✔	✔	✔	✔
FM2	✔	X	✔	✔	✔	X
CSM	✔	✔	✔	✔	✔	✔
SSM	✔	X	✔	✔	✔	✔
GSM	X	X	X	✔	X	X
GSF	X	X	X	✔	X	X

These residues could become an extra income, also helping minimize waste disposal, and be used as a technological ingredient for food formulation. Therefore, the recommendation to apply these oilseed by-products as ingredients for food industry formulations, healthy diets, and human consumption is very incentivized.

CHAPTER V

5 INFLUENCE OF FOOD PROCESSING ON THE FUNCTIONAL PROPERTIES AND PROTEIN QUALITY OF OILSEED BY-PRODUCTS

Several studies showed that food processing techniques could improve the nutritional quality of plant proteins and eliminate the compounds that impair protein digestibility (e.g., antinutritional factors). However, conventional thermal methods using high temperatures for long times may also bring some drawbacks, such as losses of desirable compounds, like reducing vitamins and minerals assimilation, and the essential amino acids bioavailability. Alternatively, some processing technologies have been investigated for the best protein employment, such as ultrasound and microwave, which can be used for valorizing plant-based proteins and agro-industrial residues and contribute to environmental preservation by reducing wastewater production, organic solvents utilization, and processing time.

Regarding the results of Chapter 4, pumpkin seed, flaxseed, and sesame seed meals were selected as the protein sources for this chapter. The aim is the evaluation of processing (cooking, microwave, and ultrasound) influence on the in vitro protein digestibility (IVPD), presence of antinutritional factors (ANFs), the amino acid (AA) profile and score (AAS), and the functional properties of the oilseed by-products.

5.1 MATERIALS AND METHODS

5.1.1 Sample collection

Raw shelled pumpkin seeds (*Cucurbita moschata,* cultivated in China), brown flaxseeds (*Linum usitatissimum*, cultivated in Brazil), and white peeled sesame seeds (*Sesamum indicum L*. cultivated in India) were purchased at a natural grocery store (Mundo Cerealista Comércio de Alimentos LTDA., Brazil). The oilseed meals were produced employing cold-pressing extraction to obtain oil from the seed, without organic solvents, using an automatic oil extractor equipment (model YJ-110, Eurolume Iluminação e Decoração Eireli, Brazil). The temperature of the oil extraction process was 49 ± 4 ºC. The samples were homogenized using sieves (80 mesh) and stored at -18 ºC for further analyses.

5.1.2 Experimental design

Response surface methodology (RSM) and a central composite design (CCD) were used to evaluate the influence of three independent processing parameters (variables) temperature (X1), pH (X2), and time (X3) on the IVPD (dependent variable). The measured dependent variable (Y, IVPD%) fit as a function of the coded independent variables (X*i*) was evaluated using a polynomial equation. Factors levels were selected according to preliminary experiments and based on an extensive literature review on the food processing influence on the nutritional quality of plant proteins (SÁ; MORENO; CARCIOFI, 2019).

5.1.3 Food processing

Cooking processing was conducted using water-bath equipment. Microwave processing was performed using a microwave reactor (model Monowave200, Anton Paar®, Brazil) operating at a maximum power of 850 W. Ultrasound was conducted using an ultrasound bath (model USC 1400A, Unique®, Brazil) with a frequency of 40 kHz and power density of 135 W/L. The oilseed samples (6.25 mg protein/mL, total volume 10 mL) were placed in transparent plastic containers (polyethylene, 5 cm x 23 cm) and processed at pre-set parameters according to the experimental CCD: temperature between 40 – 100 ºC; pH values between 5.32 – 8.68; and processing time of 5 – 45 min.

5.1.4 Analytical methods

5.1.4.1 Proximate composition

The proximate analysis of the raw oilseed by-products was carried out using official AOAC procedures (2012) for moisture (method 925.09), ash (923.03), lipids (920.39), nitrogen (954.01), and crude fiber (962.09), as described previously in Section 4.1.3.1.

5.1.4.2 In vitro protein digestibility (IVPD)

The evaluation of the IVPD of the raw and processed oilseed samples was performed using Hsu et al. (1977) method as previously described in Section 4.1.3.3.

5.1.4.3 Amino acid composition

The processed samples were freeze-dried using a laboratory freeze-dryer (model LD101, Liotop®, Brazil) and stored at -18 ºC. The determination of total amino acids of the oilseed meals was performed by reverse phase column (C18 from Phenomenex) chromatography in a high-performance liquid chromatograph (HPLC, SHIMADZU®) as described previously in Section 4.1.3.4, using Hagen et al. (1989) and White et al. (1986) methods. Tryptophan was destroyed by acid hydrolysis and was spectrophotometrically determined (590 nm) (SPIES, 1967) after enzymatic hydrolysis using pronase at 40 °C for 22 h followed by a colorimetric reaction with 4-dimethylaminobenzaldehyde (DAB) in 21.1 N sulfuric acid.

5.1.4.4 Amino acid score and in vitro protein digestibility-correct amino acid score (IVPDCAAS)

The evaluation of the AAS and IVPDCAAS of the raw and processed oilseed samples was performed as previously described in Section 4.1.3.5.

5.1.4.5 Antinutritional factors

The antinutritional factors' presence was evaluated, determining trypsin inhibition activity (TIA), tannin concentration, and phytic acid content. They were spectrophotometrically determined as described in Section 4.1.3.2. The Kakade et al. (1974) method evaluated the TIA, expressed as the trypsin inhibition unit (TIU) per milligram of the sample. The tannin content was estimated by the colorimetric method of vanillin/HCl (BURNS, 1971), and the concentration was expressed in mg of catechin per gram of sample. The Haug and Lantzsch (1983) method evaluated the phytic acid content, expressed as µg of phytate per gram of sample.

5.1.5 Functional properties

5.1.5.1 Protein solubility in the plant matrix

Protein solubility (PS) of the raw and processed meals were measured according to the Vogelsang-O'Dwyer et al. (2020) method with some modifications. Dispersions of 1% (w/v) of protein were prepared, and the pH was adjusted to the desired value (3 – 9) with 0.1 M HCl or 0.1 N NaOH. Then, the sample suspensions were stirred overnight at room temperature. After, these suspensions were centrifuged at maximum speed (4893×g) (model K14-5000M, KASVI®, Brazil) for 20 min to obtain the supernatants. The supernatant protein content was measured using the Kjeldahl method (954.01, AOAC, 2012) and 6.25 as the conversion factor. PS was calculated as the protein ratio contained in the supernatant to the original sample protein content.

5.1.5.2 Foaming capacity and stability in the plant matrix

The raw and processed meals foaming capacity (FC) and foam stability (FS) were determined as described by Liu et al. (2018) with minor modifications. Protein dispersions (50 mg) were prepared with 10 mL of phosphate buffer (0.01 M, pH 7) (initial liquid volume; V_0). The sample suspensions were homogenized with an Ultra-Turrax (model T25, IKA®, Brazil) for 2 min and poured into 50 mL graduated cylinders. The foam volume was recorded at the start (V_1) and after 30 min (V_2). The following equations calculated FC and FS:

$$FC\ (\%) = \frac{V_1 - V_0}{V_0} \times 100 \quad (2)$$

$$FS\ (\%) = \frac{V_2 - V_0}{V_0} \times 100 \quad (3)$$

5.1.5.3 Water- and oil-holding capacity in the plant matrix

Samples water-holding capacity (WHC) and oil-holding capacity (OHC) were determined by the method of Stone et al. (2015) with some modifications. Protein suspensions (0.5 g) were mixed with soybean oil or distilled water (5 g) in a 50 mL pre-weighed centrifuge tube. Samples were vortexed for 1 min every 5 min six times and, then, centrifuged (model K14-5000M, KASVI®, Brazil) at maximum speed (4893×g) for 15 min. The supernatant was

carefully decanted, the excess oil/water in the upper phase was drained for 30 min, and the remaining samples were weighed. The WHC and OHC were determined as the water/oil absorbed per gram of sample, calculated by dividing the sample weight gained by the original weight.

5.1.6 Statistical analysis

The software Statistica® (v.13.5, Statsoft Inc.) was used to perform the experimental data statistical analysis, adopting a confidence level of 95% in all cases. The Tukey's test was used to compare the ANFs, IVPD, AA profile, AAS, and functional properties (PS, FC, FS, WHC, and OHC) of raw and processed oilseed meals. Results are expressed as mean ± standard deviation of replicated samples.

5.2 RESULTS AND DISCUSSIONS

5.2.1 Proximate composition

Table 11 shows that pumpkin seed, flaxseed, and sesame seed are rich sources of lipids (53.2%, 42.6%, and 52.6%, respectively) and proteins (32.3%, 21.3%, and 22.5%), which contributes to a high energy value (637.8, 564.4, and 596.3 kcal/100g). These results were similar to that reported in the literature for the same oilseeds (KOTECKA-MAJCHRZAK et al., 2020). An increase of proteins and carbohydrates was observed for the meals compared to the raw shelled pumpkin seeds, brown flaxseeds, and white peeled sesame seeds. However, a decrease of 19.9%, 80.0%, and 13.9% in the lipids for the pumpkin seed, flaxseed, and sesame seed meals, respectively, was expected after oil extraction.

The mechanical screw pressing produces high-quality oils and meals compared to conventional extractions since using solvent, and high temperatures can lead to oil darkening and degradation of minor thermosensitive components (MACIEL et al., 2020). This cold-pressing extraction provided 69.0%, 54.3%, and 52.6% of oil yield for pumpkin seed, flaxseed, and sesame seed, respectively. PSM and SSM lipids results demonstrated that a great amount of oil residual is still present in the samples, diluting other nutrients' concentration in the proximate composition.

Table 11 – Proximate composition and energy values of oilseeds and meals (raw samples).

Proximate composition	Pumpkin seed[1]	PSM	Flaxseed[1]	FSM	Sesame seed[1]	SSM
Moisture (%)	7.2	7.0 ± 0.2	6.0	6.6 ± 0.1	5.0	4.7 ± 0.3
% Dry weight basis						
Ash	6.0	6.3 ± 0.1	5.0	5.7 ± 0.1	4.1	4.5 ± 0.1
Lipids	53.2	42.6 ± 0.9	42.6	8.5 ± 0.3	52.6	45.3 ± 0.4
Protein[2]	32.3	44.4 ± 0.5	21.3	38.8 ± 0.4	22.5	35.2 ± 0.7
Crude fiber	1.0	0.5 ± 0.1	7.1	6.4 ± 0.1	2.0	4.5 ± 0.9
Carbohydrate[3]	7.5	6.2	24.1	40.6	8.2	10.5
Energy (kcal/100g)	637.8	585.8	564.4	394.1	596.3	590.5
% Dry weight and lipid-free basis						
Ash	12.8	11.0	8.7	6.8	8.6	8.2
Protein	69.0	77.4	37.1	46.5	47.5	64.4
Crude fiber	2.1	0.9	12.4	7.7	4.2	8.2
Carbohydrate	16.0	10.8	42.0	44.4	17.3	19.2

All values are means ± standard deviation. PSM = pumpkin seed meal; FSM = flaxseed meal; SSM = sesame seed meal.

[1] Information provided by the seed manufacturer.

[2] N x 6.25.

[3] The available carbohydrate content was determined by calculating the percentile difference from all the other constituents according to the formula: [100 g dry weight – (g crude protein + g lipids + g ash + g crude fiber)].

The oil residual factor elimination will increase the concentration of the other constituents (protein, ash, carbohydrate and crude fiber). Thus, these nutrients were also calculated on a lipid-free basis and are presented in Table 11.

However, the residual lipids presented in the oilseed meals have great potential as a source of fatty acids and bioactive metabolites, with the presence of significant amounts of carotenoids, phenolic compounds, tocopherols, and phytosterols in pumpkin seeds (VERONEZI; JORGE, 2012; RABRENOVIĆ et al., 2014); linolenic and linoleic acid, and lignans in flaxseeds (SHIM et al., 2014); and phytosterols, polyunsaturated fatty acids, tocopherols, and lignans (e.g., phenylpropanoid) in sesame seeds (PATHAK et al., 2014).

Additionally, in terms of dietary fibers, a high source contains > 6 g/100 g (WHO, 2004); therefore, flaxseed meal presented high amounts of dietary fibers (6.4 g/100 g) and is considered a high dietary fiber source. In terms of carbohydrates, flaxseed presents a high amount of this macronutrient, and the polysaccharide gum present is this source has been traditionally used as an egg white substitute in food formulations, such as bakery and ice cream products (IZYDORCZYK; CUI; WANG, 2005). Furthermore, the protein results for the oilseed meals (35.2% – 44.4%) are comparable to other oilseed meals, such as rapeseed (32.8%) (JIA et al., 2021) and black mustard seed (38.2%) (SARKER et al., 2015), and also comparable to traditional plant protein sources in the human diet (soybean, peas, and common beans), which present protein content of 35.3%, 21.7%, and 19.9% respectively (TERRIEN, 2017). Thus, these results show the potential of these oilseed by-products as protein sources in food formulations and human nutrition.

5.2.2 In vitro protein digestibility (IVPD)

IVPD is a suitable tool for evaluating the nutritional quality of a food protein, combined with the amino acid composition and its bioavailability (SÁ; MORENO; CARCIOFI, 2019). The IVPD for the unprocessed samples was 85.5 ± 1.7% (PSM), 88.4 ± 0.1% (FSM), and 88.9 ± 1.5% (SSM), without pH adjustment (pH 6.33, pH 5.91, and pH 6.90, respectively). Thus, the process parameters – temperature, pH, and processing time – were evaluated in the IVPD response for all meals, regarding cooking, microwave, and ultrasound processes, using an experimental design. The results obtained after 17 trials for each process and seed meal are shown in Tables 12 – 14. Coefficients of adjusted polynomial models and their analysis of variance (ANOVA) results were calculated and are shown in

Tables 15 – 17. The regression equations demonstrate an empirical relationship between the in vitro protein digestibility and the studied variables in coded units. Responses surfaces plots of independent variables on IVPD are presented in Figures 3 – 5.

Overall, the regression model developed after ANOVA was significant ($p < 0.05$) for all treatments and seed meals with an insignificant lack of fit, which confirmed that the developed model could adequately represent the real relationship among the chosen parameters. Results for processed PSM indicated that all independent linear factors (temperature, pH, and time) positive significantly ($p < 0.05$) affected the IVPD for microwave and cooking processes. However, the processing time (linear factor) did not significantly affect the IVPD ($p < 0.05$) for the ultrasound process. For FSM, all independent linear factors (temperature, pH, and time) positive significantly ($p < 0.05$) affected the IVPD for all processes. Although the coefficient of correlation (R) for PSM and FSM responses was relatively low (0.80 – 0.83), the applied regression models were adequate.

Regarding the results for processed SSM, ANOVA indicated that all independent linear factors (temperature, pH, and time) significantly ($p < 0.05$) affected the IVPD for ultrasound and cooking processes. However, the processing time did not significantly affect the IVPD ($p < 0.05$) for the microwave. Besides, the coefficient of correlation (R) for all SSM responses was higher than 0.93, which implies the adequacy of the applied regression models. IVPD results, presented in Tables 12 – 14, for all oilseed meals, ranged between 83.7% and 96.1%, and the best parameters among all studied processes were 87.8ºC, pH 8.00, and process time of 37 min.

As seen in the response surfaces, regarding the mutual effects of the independent variables on the PSM IVPD responses for cooking and microwave processing, the IVPD increased as temperature, time, and pH values increased (Figure 3). Figure 3 also showed that IVPD was highly dependent on the pH values and temperature for ultrasound processing, reaching the highest response at high-temperature levels and low dependent on the processing time, within the time range used, which can be maintained at low levels for industrial economic viability. For FSM, shown in Figure 4, the IVPD responses for all processes were highly dependent on temperature, time, and pH values. Regarding the SSM IVPD responses for cooking and ultrasound processing, the IVPD increased as temperature, time, and pH values increased (Figure 5).

Table 12 – Experimental central composite 2^3-factorial design matrix and in vitro protein digestibility responses for cooking, microwave, and ultrasound treatments (pumpkin seed by-products).

Runs	x1, Temperature (ºC)	x2, pH	x3, Time (min)	IVPD% Cooking	IVPD% Microwave	IVPD% Ultrasound
1	-1 (52.2)	-1 (6.00)	-1 (13)	83.94	83.76	83.67
2	-1 (52.2)	-1 (6.00)	+1 (37)	84.12	84.67	83.94
3	-1 (52.2)	+1 (8.00)	-1 (13)	91.00	92.81	90.91
4	-1 (52.2)	+1 (8.00)	+1 (37)	91.72	93.35	91.00
5	+1 (87.8)	-1 (6.00)	-1 (13)	83.76	85.57	86.11
6	+1 (87.8)	-1 (6.00)	+1 (37)	84.30	86.11	86.84
7	+1 (87.8)	+1 (8.00)	-1 (13)	92.09	95.71	92.27
8	**+1 (87.8)**	**+1 (8.00)**	**+1 (37)**	**94.08**	**96.07**	**93.08**
9	-1.682 (40.0)	0 (7.00)	0 (25)	83.76	83.76	83.81
10	+1.682 (100.0)	0 (7.00)	0 (25)	90.10	90.10	91.00
11	0 (70.0)	-1.682 (5.32)	0 (25)	83.76	84.30	84.67
12	0 (70.0)	+1.682 (8.68)	0 (25)	85.57	86.11	86.02
13	0 (70.0)	0 (7.00)	-1.682 (5)	83.76	83.76	83.81
14	0 (70.0)	0 (7.00)	+1.682 (45)	84.30	84.48	84.39
15	0 (70.0)	0 (7.00)	0 (25)	85.03	84.48	84.67
16	0 (70.0)	0 (7.00)	0 (25)	85.39	84.67	84.85
17	0 (70.0)	0 (7.00)	0 (25)	85.21	84.85	85.12

Table 13 – Experimental central composite 2^3-factorial design matrix and in vitro protein digestibility responses for cooking, microwave, and ultrasound treatments (flaxseed by-products).

Runs	x1, Temperature (ºC)	x2, pH	x3, Time (min)	IVPD% Cooking	IVPD% Microwave	IVPD% Ultrasound
1	-1 (52.2)	-1 (6.00)	-1 (13)	86.66	86.84	86.93
2	-1 (52.2)	-1 (6.00)	+1 (37)	87.56	87.38	87.83
3	-1 (52.2)	+1 (8.00)	-1 (13)	90.64	90.46	92.81
4	-1 (52.2)	+1 (8.00)	+1 (37)	92.09	91.72	93.17
5	+1 (87.8)	-1 (6.00)	-1 (13)	88.10	88.47	88.83
6	+1 (87.8)	-1 (6.00)	+1 (37)	90.64	89.19	89.55
7	+1 (87.8)	+1 (8.00)	-1 (13)	92.99	92.81	92.90
8	**+1 (87.8)**	**+1 (8.00)**	**+1 (37)**	**94.98**	**93.72**	**93.99**
9	-1.682 (40.0)	0 (7.00)	0 (25)	86.48	86.29	86.57
10	+1.682 (100.0)	0 (7.00)	0 (25)	93.35	91.54	92.09
11	0 (70.0)	-1.682 (5.32)	0 (25)	86.93	87.02	87.11
12	0 (70.0)	+1.682 (8.68)	0 (25)	87.38	87.74	87.47
13	0 (70.0)	0 (7.00)	-1.682 (5)	86.66	86.84	86.93
14	0 (70.0)	0 (7.00)	+1.682 (45)	87.11	87.38	87.20
15	0 (70.0)	0 (7.00)	0 (25)	87.47	87.02	87.56
16	0 (70.0)	0 (7.00)	0 (25)	87.56	87.20	87.65
17	0 (70.0)	0 (7.00)	0 (25)	87.74	87.38	87.83

Table 14 – Experimental central composite 2^3-factorial design matrix and in vitro protein digestibility responses for cooking, microwave, and ultrasound treatments (sesame seed by-products).

Runs	x1, Temperature (ºC)	x2, pH	x3, Time (min)	IVPD% Cooking	IVPD% Microwave	IVPD% Ultrasound
1	-1 (52.2)	-1 (6.00)	-1 (13)	87.56	88.29	87.65
2	-1 (52.2)	-1 (6.00)	+1 (37)	88.00	88.83	88.74
3	-1 (52.2)	+1 (8.00)	-1 (13)	91.18	91.00	91.91
4	-1 (52.2)	+1 (8.00)	+1 (37)	92.90	93.17	92.81
5	+1 (87.8)	-1 (6.00)	-1 (13)	90.10	89.73	90.46
6	+1 (87.8)	-1 (6.00)	+1 (37)	90.64	90.10	90.91
7	+1 (87.8)	+1 (8.00)	-1 (13)	94.08	93.90	93.17
8	**+1 (87.8)**	**+1 (8.00)**	**+1 (37)**	**95.25**	**94.44**	**94.71**
9	-1.682 (40.0)	0 (7.00)	0 (25)	87.29	87.56	87.74
10	+1.682 (100.0)	0 (7.00)	0 (25)	92.45	92.63	92.54
11	0 (70.0)	-1.682 (5.32)	0 (25)	87.56	87.38	87.83
12	0 (70.0)	+1.682 (8.68)	0 (25)	91.91	92.81	91.80
13	0 (70.0)	0 (7.00)	-1.682 (5)	88.47	88.83	88.74
14	0 (70.0)	0 (7.00)	+1.682 (45)	90.00	89.91	90.64
15	0 (70.0)	0 (7.00)	0 (25)	90.82	90.64	90.69
16	0 (70.0)	0 (7.00)	0 (25)	90.46	91.00	90.71
17	0 (70.0)	0 (7.00)	0 (25)	90.10	91.54	90.42

Table 15 – Variance analysis (ANOVA) of processes parameters (temperature, pH, and time) on IVPD of pumpkin seed meals.

Processes parameters	Degree of freedom	Mean sum of squares	F-value	p-value	Significance	Coefficient of correlation (R)	Model
Ultrasound						0.83	$IVPD\% = 84.70 + 1.53x1 + 1.52x1^2 + 2.12x2 + 0.79x2^2 + 0.35x3^2$
(1) Temperature (L.)	1	31.87	614.45	0.0016	S		
(1) Temperature (Q.)	1	26.03	501.84	0.0020	S		
(2) pH (L.)	1	61.50	1185.59	0.0008	S		
(2) pH (Q.)	1	7.04	135.65	0.0073	S		
(3) Time (L.)	1	0.61	11.67	0.0761	NS		
(3) Time (Q.)	1	1.39	26.88	0.0352	S		
1L by 2L	1	0.45	8.70	0.0983	NS		
1L by 3L	1	0.17	3.34	0.2093	NS		
2L by 3L	1	0.00	0.02	0.9011	NS		
Microwave						0.80	$IVPD\% = 84.39 + 1.43x1 + 1.75x1^2 + 2.99x2 + 1.14x2^2 + 0.26x3 + 0.76x3^2 + 0.29x1x2$
(1) Temperature (L.)	1	27.91	851.91	0.0012	S		
(1) Temperature (Q.)	1	34.62	1056.82	0.0009	S		
(2) pH (L.)	1	122.33	3733.93	0.0003	S		
(2) pH (Q.)	1	14.77	450.78	0.0022	S		
(3) Time (L.)	1	0.93	28.50	0.0333	S		
(3) Time (Q.)	1	6.52	199.06	0.0050	S		
1L by 2L	1	0.69	21.13	0.0442	S		
1L by 3L	1	0.04	1.13	0.4000	NS		
2L by 3L	1	0.04	1.13	0.4000	NS		
Cooking						0.80	$IVPD\% = 85.02 + 1.03x1 + 1.26x1^2 + 2.62x2 + 0.45x2^2 + 0.32x3 + 0.23x3^2 + 0.42x1x2$
(1) Temperature (L.)	1	14.54	443.92	0.0022	S		
(1) Temperature (Q.)	1	17.78	542.75	0.0018	S		
(2) pH (L.)	1	93.87	2865.37	0.0003	S		
(2) pH (Q.)	1	2.34	71.54	0.0137	S		
(3) Time (L.)	1	1.39	42.34	0.0228	S		
(3) Time (Q.)	1	0.61	18.52	0.0510	S		
1L by 2L	1	1.48	45.13	0.0215	S		
1L by 3L	1	0.33	10.13	0.0862	NS		
2L by 3L	1	0.50	15.13	0.0602	NS		

S – There is significant effect of process parameter on response variable (IVPD%) at 5% significant level ($p < 0.05$).
NS – There is no significant effect of process parameter on response variable (IVPD%) at 5% significant level ($p < 0.05$).

Table 16 – Variance analysis (ANOVA) of processes parameters (temperature, pH, and time) on IVPD of flaxseed meals.

Processes parameters	Degree of freedom	Mean sum of squares	F-value	p-value	Significance	Coefficient of correlation (R)	Model
Ultrasound						0.80	$IVPD\% = 87.50 + 1.01x1 + 1.21x1^2 + 1.49x2 + 0.49x2^2 + 0.26x3 + 0.41x3^2 - 0.34x1x2$
(1) Temperature (L.)	1	13.96	730.67	0.0014	S		
(1) Temperature (Q.)	1	16.59	867.94	0.0012	S		
(2) pH (L.)	1	30.29	1584.83	0.0006	S		
(2) pH (Q.)	1	2.74	143.41	0.0069	S		
(3) Time (L.)	1	0.91	47.84	0.0203	S		
(3) Time (Q.)	1	1.92	100.65	0.0098	S		
1L by 2L	1	0.92	48.21	0.0201	S		
1L by 3L	1	0.04	1.93	0.2994	NS		
2L by 3L	1	0.00	0.21	0.6889	NS		
Microwave						0.82	$IVPD\% = 87.06 + 1.22x1 + 1.10x1^2 + 1.32x2 + 0.55x2^2 + 0.32x3 + 0.46x3^2$
(1) Temperature (L.)	1	20.20	616.69	0.0016	S		
(1) Temperature (Q.)	1	13.73	419.23	0.0024	S		
(2) pH (L.)	1	23.86	728.24	0.0014	S		
(2) pH (Q.)	1	3.53	107.85	0.0091	S		
(3) Time (L.)	1	1.39	42.34	0.0228	S		
(3) Time (Q.)	1	2.43	74.03	0.0132	S		
1L by 2L	1	0.10	3.13	0.2191	NS		
1L by 3L	1	0.00	0.13	0.7575	NS		
2L by 3L	1	0.10	3.13	0.2191	NS		
Cooking						0.83	$IVPD\% = 87.43 + 1.56x1 + 1.37x1^2 + 1.35x2 + 0.39x2^2 + 0.56x3 + 0.29x3^2 + 0.27x1x3$
(1) Temperature (L.)	1	33.35	1745.10	0.0006	S		
(1) Temperature (Q.)	1	21.01	1099.34	0.0009	S		
(2) pH (L.)	1	25.06	1311.21	0.0008	S		
(2) pH (Q.)	1	1.71	89.37	0.0110	S		
(3) Time (L.)	1	4.27	223.59	0.0044	S		
(3) Time (Q.)	1	0.97	50.73	0.0191	S		
1L by 2L	1	0.07	3.43	0.2053	NS		
1L by 3L	1	0.59	30.86	0.0309	S		
2L by 3L	1	0.00	0.00	1.0000	NS		

S – There is significant effect of process parameter on response variable (IVPD%) at 5% significant level ($p < 0.05$).
NS – There is no significant effect of process parameter on response variable (IVPD%) at 5% significant level ($p < 0.05$).

Table 17 – Variance analysis (ANOVA) of processes parameters (temperature, pH, and time) on IVPD of sesame seed meals.

Processes parameters	Degree of freedom	Mean sum of squares	F-value	p-value	Significance	Coefficient of correlation (R)	Model
Ultrasound						0.93	$IVPD\% = 90.51 + 1.19x1 + 1.57x2 + 0.53x3$
(1) Temperature (L.)	1	19.24	731.23	0.0014	S		
(1) Temperature (Q.)	1	0.28	10.73	0.0819	NS		
(2) pH (L.)	1	33.87	1287.11	0.0008	S		
(2) pH (Q.)	1	0.02	0.79	0.4670	NS		
(3) Time (L.)	1	3.77	143.36	0.0069	S		
(3) Time (Q.)	1	0.00	0.00	0.9745	NS		
1L by 2L	1	0.41	15.56	0.0587	NS		
1L by 3L	1	0.00	0.00	1.0000	NS		
2L by 3L	1	0.10	3.89	0.1873	NS		
Microwave						0.94	$IVPD\% = 90.97 + 1.13x1 + 1.81x2$
(1) Temperature (L.)	1	17.37	83.71	0.0117	S		
(1) Temperature (Q.)	1	0.01	0.05	0.8436	NS		
(2) pH (L.)	1	44.67	215.27	0.0046	S		
(2) pH (Q.)	1	0.01	0.05	0.8436	NS		
(3) Time (L.)	1	2.17	10.47	0.0837	NS		
(3) Time (Q.)	1	0.92	4.46	0.1692	NS		
1L by 2L	1	0.26	1.26	0.3778	NS		
1L by 3L	1	0.41	1.97	0.2952	NS		
2L by 3L	1	0.41	1.97	0.2952	NS		
Cooking						0.93	$IVPD\% = 90.35 + 1.40x1 + 1.79x2 + 0.47x3$
(1) Temperature (L.)	1	26.72	203.87	0.0049	S		
(1) Temperature (Q.)	1	0.28	2.17	0.2789	NS		
(2) pH (L.)	1	43.70	333.44	0.0030	S		
(2) pH (Q.)	1	0.14	1.05	0.4125	NS		
(3) Time (L.)	1	3.06	23.32	0.0403	S		
(3) Time (Q.)	1	0.05	0.37	0.6063	NS		
1L by 2L	1	0.00	0.01	0.9501	NS		
1L by 3L	1	0.02	0.18	0.7127	NS		
2L by 3L	1	0.46	3.51	0.2018	NS		

S – There is significant effect of process parameter on the response variable (IVPD%) at 5% significant level ($p < 0.05$).
NS – There is no significant effect of process parameter on the response variable (IVPD%) at 5% significant level ($p < 0.05$).

Figure 3 – Response surface plots of independent variables on in vitro protein digestibility (%) for pumpkin seed by-products, for ultrasound: temperature and pH (a), temperature and time (b), and pH and time (c); microwave: temperature and pH (d), temperature and time (e), and pH and time (f); and cooking: temperature and pH (g), temperature and time (h), and pH and time (i).

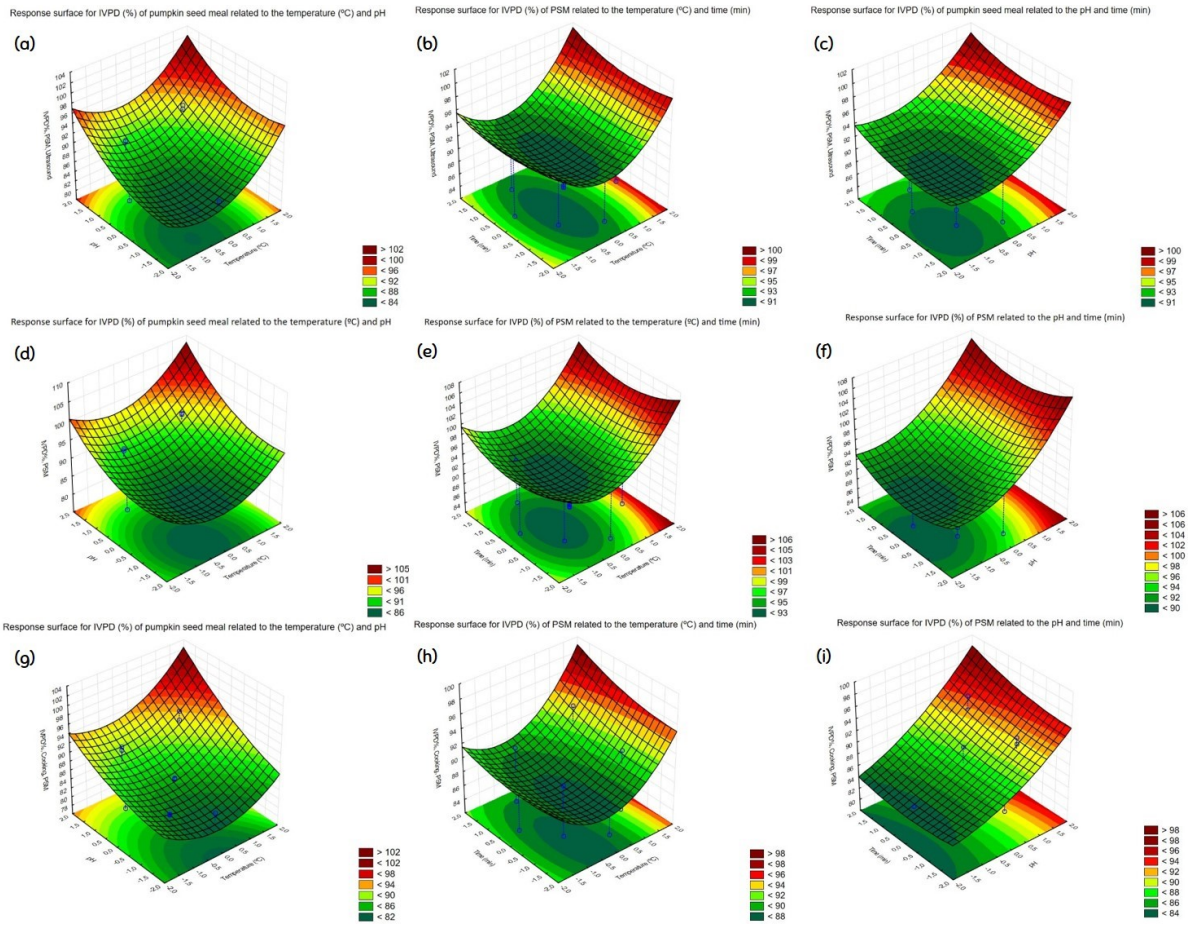


Figure 4 – Response surface plots of independent variables on in vitro protein digestibility (%) for flaxseed by-products, for ultrasound: temperature and pH (a), temperature and time (b), and pH and time (c); microwave: temperature and pH (d), temperature and time (e), and pH and time (f); and cooking: temperature and pH (g), temperature and time (h), and pH and time (i).

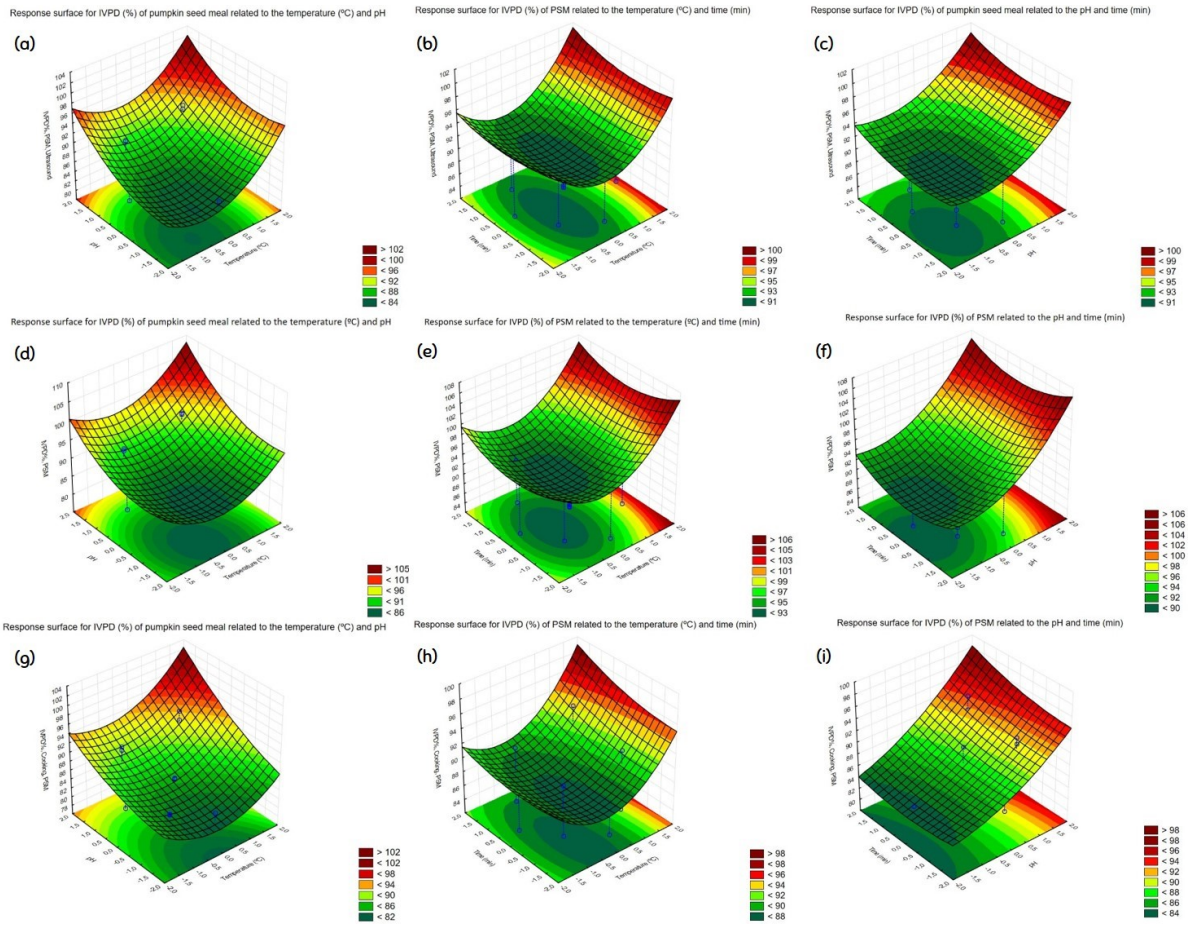


Figure 5 – Response surface plots of independent variables on in vitro protein digestibility (%) for sesame seed by-products, for ultrasound: temperature and pH (a), temperature and time (b), and pH and time (c); microwave: temperature and pH (d), temperature and time (e), and pH and time (f); and cooking: temperature and pH (g), temperature and time (h), and pH and time (i).

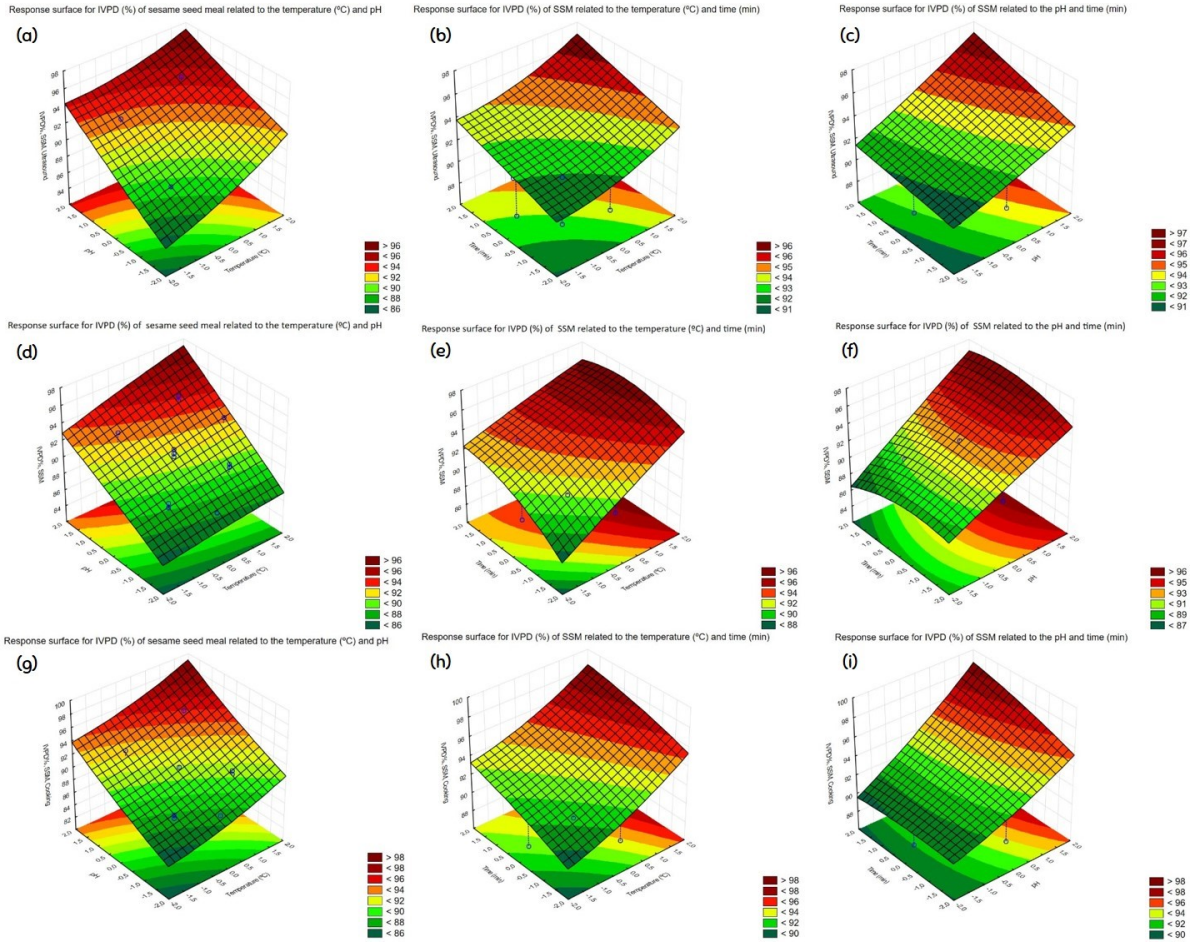


Figure 5 also showed that IVPD was highly dependent on the pH values and temperature for microwave processing, reaching the highest response at high-temperature levels and low dependent on the processing time. These findings agree with Görgüç et al. (2019), where increasing pH values increased protein extraction from sesame bran. As the oilseed samples were subjected to processing, a change in the protein conformation (secondary and tertiary structure) would reduce its susceptibility to the digestive enzymes, but as the processing time increased, the protein would denature, and digestion would proceed as desired (VAGADIA et al., 2018). Several researchers have proposed that the treatment temperature is the key determinant of food protein digestibility. As used in this work (87.8 ºC), a relatively high temperature improves protein quality while inactivating the compounds that lower the protein digestibility of plant proteins (ANFs) (SÁ; MORENO; CARCIOFI, 2019). The proper heat process can affect the conformational properties of food proteins (tertiary and secondary structure) and accelerate their denaturation without changing their primary structure or reducing protein solubility. During the hydrolytic process, the protein molecules unfold and become more accessible to proteases than in their native state (LI et al., 2010), impacting (positively or not) the protein digestibility and the amino acid profile. Several authors demonstrated that thermal processing increases the reduction and inactivation of antinutritional factors, such as protease inhibitors, tannins, fibers, and phytic acid (SÁ; MORENO; CARCIOFI, 2019).

Furthermore, high temperatures synergy with ultrasound processing can enhance mass transfer, providing high shear forces in the food matrix, modifying proteins by affecting H-bonds, reducing protein aggregates, and improving protein functionality (GÖRGÜÇ; BIRCAN; YILMAZ, 2019). This influence in the amino acid profile, ANFs concentration, and functional properties of processed sesame seed meals is discussed in Sections 5.2.3, 5.2.4, and 5.2.5, respectively. Additionally, pH 8.0 was the best parameter value according to the experimental design. The results of protein solubility (pH-dependent) for the processed oilseed meals, as discussed in Section 5.2.5.1, can help explain the influence of pH in the increasing IVPD.

Finally, the time process is a crucial parameter. Overheating proteins may depress digestibility and amino acid availability, causing a slower release of amino acids from the protein and decomposition of essential amino acids. Therefore, a safe heating process is critical to processing plant proteins to establish maximum nutritional value (SÁ; MORENO; CARCIOFI, 2019). Table 18 summarizes the IVPD results for the raw and processed oilseed meals.

Table 18 – Influence of processing on the in vitro protein digestibility of oilseed meals in this study.

Source of plant protein	Food processing	Protein quality evaluation method	Results
Pumpkin seed meal	Raw, pH 6.33	IVPD (%)	85.50
	Cooking (87.8 ºC, pH 8, 37 min)		94.08
	Microwave (87.8 ºC, pH 8, 37 min)		96.07
	Ultrasound (87.8 ºC, pH 8, 37 min)		93.08
Flaxseed meal	Raw, pH 5.91	IVPD (%)	88.40
	Cooking (87.8 ºC, pH 8, 37 min)		94.98
	Microwave (87.8 ºC, pH 8, 37 min)		93.72
	Ultrasound (87.8 ºC, pH 8, 37 min)		93.99
Sesame seed meal	Raw, pH 6.90	IVPD (%)	88.90
	Cooking (87.8 ºC, pH 8, 37 min)		95.25
	Microwave (87.8 ºC, pH 8, 37 min)		94.44
	Ultrasound (87.8 ºC, pH 8, 37 min)		94.71

5.2.3 Amino acid composition, amino acid score, and in vitro protein digestibility-correct amino acid score (IVPDCAAS))

The amino acid (AA) composition of the raw and processed samples of pumpkin seed, flaxseed, and sesame seed meals is presented in Table 19 – 21. The total AA content for the samples shows similarity to those results presented for protein content (Table 11), which corroborates the analysis's veracity.

These findings agree with previous studies and literature review (SÁ; MORENO; CARCIOFI, 2020; SÁ et al., 2021), reporting the amino acid profile of oilseeds sources and by-products. The results of AA composition of PSM raw sample (Table 19) presented statistical differences ($p < 0.05$) between all processing treatments for each amino acid evaluated, except lysine (for cooking and microwave), threonine (for cooking), aspartic acid (for microwave and ultrasound), and aromatic amino acids (for microwave). Total AA content was reduced 2.5%, 3.1%, and 2.9%, for cooking, microwave, and ultrasound, respectively; for essential amino acids (EAA), the concentration decreased 4.1%, 3.3%, and 4.1%, when compared to the raw sample. Also, the results showed that the major reductions in amino acid concentration regarding all processing for PSM were tryptophan (32 – 38%), sulfur amino acids (Met + Cys, ~25%), and isoleucine (~23%).

Table 19 – Amino acid composition, Amino Acid Score for adults, and IVPDCAAS of the raw and processed pumpkin seed meals.

AA composition (g/100g protein)	Requirement pattern[1] (g/100g protein)	Raw Sample		Cooking		Microwave		Ultrasound	
		AA	AAS[2] (%)	AA	AAS[2] (%)	AA	AAS[2] (%)	AA	AAS[2] (%)
Essential (EAA)									
Histidine (His)	1.5	2.31 ± 0.03^{A}	154.2 ± 1.9^{a}	2.35 ± 0.01^{B}	156.8 ± 0.4^{b}	2.36 ± 0.01^{B}	157.6 ± 0.5^{b}	2.35 ± 0.02^{B}	156.9 ± 1.0^{b}
Isoleucine (Ile)	3.0	4.28 ± 0.05^{C}	142.6 ± 1.7^{c}	3.42 ± 0.01^{B}	114.0 ± 0.4^{b}	3.41 ± 0.01^{B}	113.8 ± 0.2^{b}	3.30 ± 0.01^{A}	109.9 ± 0.2^{a}
Leucine (Leu)	5.9	6.44 ± 0.03^{A}	109.2 ± 0.5^{a}	7.50 ± 0.01^{C}	127.1 ± 0.1^{c}	7.53 ± 0.01^{D}	127.7 ± 0.2^{d}	7.38 ± 0.01^{B}	125.1 ± 0.1^{b}
Lysine (Lys)	4.5	3.89 ± 0.10^{B}	86.5 ± 2.2^{b}	3.88 ± 0.01^{AB}	86.2 ± 0.1^{ab}	3.87 ± 0.01^{AB}	86.1 ± 0.1^{ab}	3.80 ± 0.01^{A}	84.4 ± 0.1^{a}
Threonine (Thr)	2.3	2.97 ± 0.02^{B}	129.3 ± 1.0^{b}	2.95 ± 0.08^{B}	128.2 ± 3.3^{b}	2.80 ± 0.01^{A}	121.9 ± 0.1^{a}	2.78 ± 0.01^{A}	120.7 ± 0.1^{a}
Tryptophan (Trp)	0.6	1.27 ± 0.01^{D}	211.1 ± 0.5^{d}	0.78 ± 0.04^{A}	129.7 ± 6.2^{a}	1.07 ± 0.02^{C}	179.0 ± 2.6^{c}	0.86 ± 0.02^{B}	143.1 ± 3.6^{b}
Valine (Val)	3.9	4.90 ± 0.01^{C}	125.6 ± 0.1^{c}	4.49 ± 0.02^{B}	115.2 ± 0.4^{b}	4.48 ± 0.01^{B}	115.0 ± 0.3^{b}	4.33 ± 0.02^{A}	111.1 ± 0.4^{a}
Total sulfur amino acids (Met + Cys)	2.2	2.64 ± 0.01^{C}	119.9 ± 0.1^{c}	1.97 ± 0.01^{A}	89.3 ± 0.1^{a}	2.15 ± 0.01^{B}	97.8 ± 0.1^{b}	2.83 ± 0.01^{D}	128.6 ± 0.5^{d}
Total aromatic amino acids (Phe + Tyr)	3.8	8.26 ± 0.05^{B}	217.5 ± 1.4^{b}	8.32 ± 0.01^{C}	219.1 ± 0.3^{c}	8.26 ± 0.01^{B}	217.4 ± 0.3^{b}	8.04 ± 0.02^{A}	211.5 ± 0.4^{a}
Non-essential (NEAA)									
Alanine (Ala)	-	4.43 ± 0.05^{B}	-	4.23 ± 0.01^{A}	-	4.18 ± 0.01^{A}	-	4.20 ± 0.03^{A}	-
Arginine (Arg)	-	18.58 ± 0.06^{C}	-	16.47 ± 0.02^{A}	-	16.45 ± 0.01^{A}	-	16.55 ± 0.01^{B}	-
Aspartic acid (Asp)	-	8.83 ± 0.02^{A}	-	8.97 ± 0.03^{B}	-	8.84 ± 0.03^{A}	-	8.86 ± 0.01^{A}	-
Glutamic acid (Glu)	-	20.70 ± 0.06^{C}	-	18.42 ± 0.02^{B}	-	18.30 ± 0.01^{A}	-	18.43 ± 0.02^{B}	-
Glycine (Gly)	-	5.90 ± 0.01^{A}	-	6.44 ± 0.02^{BC}	-	6.41 ± 0.03^{B}	-	6.48 ± 0.03^{C}	-
Proline (Pro)	-	4.03 ± 0.02^{A}	-	4.40 ± 0.03^{B}	-	4.48 ± 0.02^{C}	-	4.49 ± 0.04^{C}	-
Serine (Ser)	-	5.46 ± 0.01^{D}	-	5.41 ± 0.01^{C}	-	5.38 ± 0.01^{B}	-	5.33 ± 0.01^{A}	-
Total EAA (g/100g protein)	-	37.17 ± 0.23^{C}	-	35.66 ± 0.01^{A}	-	35.96 ± 0.02^{B}	-	35.66 ± 0.02^{A}	-
Total NEAA (g/100g protein)	-	62.83 ± 0.23^{A}	-	64.34 ± 0.01^{C}	-	64.04 ± 0.02^{B}	-	64.34 ± 0.02^{C}	-
Total AA (g/100g sample)	-	44.31 ± 0.23^{B}	-	43.21 ± 0.04^{A}	-	42.95 ± 0.04^{A}	-	43.01 ± 0.19^{A}	-
First limiting amino acid	-	-	Lys	-	Lys	-	Lys	-	Lys
IVPDCAAS (%)	-	-	76.6	-	82.1	-	81.3	-	79.9

All values are means ± standard deviation. Processes were performed at 87.8 ºC, pH 8.0, and 37 min.

[1] WHO/FAO/UNU (2007) Expert Consultation Report for adults.

[2] AAS: Amino Acid Score = (mg of amino acid in 1 g of test protein/mg of amino acid in requirement pattern)×100.

[A–D] Different letters in the same row indicate a significant difference in the AA composition between samples for each amino acid ($p < 0.05$ by Tukey's test).

[a–d] Different letters in the same row indicate a significant difference in the AAS between samples for each essential amino acid ($p < 0.05$ by Tukey's test).

IVPDCAAS: In Vitro Protein Digestibility-Corrected Amino Acid Score = AAS × IVPD.

Table 20 – Amino acid composition, Amino Acid Score for adults, and IVPDCAAS of the raw and processed flaxseed meals.

AA composition (g/100g protein)	Requirement pattern[1] (g/100g protein)	Raw Sample		Cooking		Microwave		Ultrasound	
		AA	AAS[2] (%)	AA	AAS[2] (%)	AA	AAS[2] (%)	AA	AAS[2] (%)
Essential (EAA)									
Histidine (His)	1.5	2.23 ± 0.03^{A}	148.6 ± 1.9^{a}	2.30 ± 0.01^{B}	153.0 ± 0.2^{b}	2.62 ± 0.02^{C}	174.3 ± 1.0^{c}	2.71 ± 0.01^{D}	180.4 ± 0.9^{d}
Isoleucine (Ile)	3.0	4.99 ± 0.01^{C}	166.3 ± 0.4^{c}	3.73 ± 0.01^{A}	124.2 ± 0.1^{a}	3.90 ± 0.05^{B}	130.1 ± 1.6^{b}	3.74 ± 0.01^{A}	124.6 ± 0.4^{a}
Leucine (Leu)	5.9	5.70 ± 0.02^{A}	96.6 ± 0.4^{a}	6.47 ± 0.01^{D}	109.7 ± 0.2^{d}	6.45 ± 0.01^{C}	109.3 ± 0.2^{c}	6.40 ± 0.01^{B}	108.5 ± 0.1^{b}
Lysine (Lys)	4.5	3.90 ± 0.01^{A}	86.8 ± 0.3^{a}	4.03 ± 0.01^{D}	89.5 ± 0.1^{d}	4.00 ± 0.01^{C}	88.9 ± 0.3^{c}	3.97 ± 0.01^{B}	88.1 ± 0.1^{b}
Threonine (Thr)	2.3	3.72 ± 0.06^{A}	161.9 ± 2.7^{a}	3.76 ± 0.01^{A}	163.6 ± 0.1^{a}	3.74 ± 0.01^{A}	162.8 ± 0.3^{a}	3.76 ± 0.01^{A}	163.3 ± 0.4^{a}
Tryptophan (Trp)	0.6	1.38 ± 0.01^{A}	230.4 ± 2.1^{a}	1.63 ± 0.06^{B}	271.3 ± 10.2^{b}	1.31 ± 0.04^{A}	217.6 ± 6.3^{a}	1.31 ± 0.04^{A}	218.6 ± 6.6^{a}
Valine (Val)	3.9	5.29 ± 0.01^{C}	135.7 ± 0.4^{c}	4.55 ± 0.02^{B}	116.7 ± 0.4^{b}	4.57 ± 0.02^{B}	117.2 ± 0.5^{b}	4.44 ± 0.02^{A}	113.7 ± 0.4^{a}
Total sulfur amino acids (Met + Cys)	2.2	2.21 ± 0.01^{A}	100.2 ± 0.5^{a}	2.48 ± 0.01^{B}	112.9 ± 0.4^{b}	2.60 ± 0.01^{D}	118.2 ± 0.1^{d}	2.55 ± 0.01^{C}	115.8 ± 0.2^{c}
Total aromatic amino acids (Phe + Tyr)	3.8	7.32 ± 0.03^{D}	192.6 ± 0.7^{d}	7.06 ± 0.02^{C}	185.8 ± 0.5^{c}	6.95 ± 0.02^{A}	182.9 ± 0.5^{a}	7.01 ± 0.02^{B}	184.5 ± 0.5^{b}
Non-essential (NEAA)									
Alanine (Ala)	-	4.62 ± 0.03^{C}	-	4.36 ± 0.01^{A}	-	4.44 ± 0.01^{B}	-	4.45 ± 0.01^{B}	-
Arginine (Arg)	-	12.74 ± 0.10^{B}	-	10.90 ± 0.01^{A}	-	10.91 ± 0.01^{A}	-	10.91 ± 0.01^{A}	-
Aspartic acid (Asp)	-	9.73 ± 0.04^{A}	-	9.82 ± 0.01^{B}	-	10.14 ± 0.01^{D}	-	10.08 ± 0.01^{C}	-
Glutamic acid (Glu)	-	23.82 ± 0.07^{D}	-	21.19 ± 0.01^{C}	-	20.87 ± 0.04^{B}	-	20.68 ± 0.03^{A}	-
Glycine (Gly)	-	6.56 ± 0.04^{A}	-	7.37 ± 0.04^{B}	-	7.40 ± 0.05^{B}	-	7.59 ± 0.03^{C}	-
Proline (Pro)	-	4.23 ± 0.02^{A}	-	4.82 ± 0.02^{C}	-	4.73 ± 0.03^{B}	-	4.90 ± 0.03^{D}	-
Serine (Ser)	-	5.42 ± 0.02^{B}	-	5.52 ± 0.01^{C}	-	5.37 ± 0.01^{A}	-	5.52 ± 0.01^{C}	-
Total EAA (g/100g protein)	-	36.75 ± 0.13^{C}	-	36.01 ± 0.09^{AB}	-	36.14 ± 0.04^{B}	-	35.88 ± 0.01^{A}	-
Total NEAA (g/100g protein)	-	63.25 ± 0.13^{A}	-	63.99 ± 0.09^{BC}	-	63.86 ± 0.04^{B}	-	64.12 ± 0.01^{C}	-
Total AA (g/100g sample)	-	39.94 ± 0.02^{C}	-	37.12 ± 0.05^{B}	-	34.58 ± 0.03^{A}	-	37.20 ± 0.08^{B}	-
First limiting amino acid	-	-	Lys	-	Lys	-	Lys	-	Lys
IVPDCAAS (%)	-	-	76.7	-	85.0	-	83.3	-	82.8

All values are means ± standard deviation. Processes were performed at 87.8 ºC, pH 8.0, and 37 min.

[1] WHO/FAO/UNU (2007) Expert Consultation Report for adults.

[2] AAS: Amino Acid Score = (mg of amino acid in 1 g of test protein/mg of amino acid in requirement pattern)×100.

$^{A–D}$ Different letters in the same row indicate a significant difference in the AA composition between samples for each amino acid ($p < 0.05$ by Tukey's test).

$^{a–d}$ Different letters in the same row indicate a significant difference in the AAS between samples for each essential amino acid ($p < 0.05$ by Tukey's test).

IVPDCAAS: In Vitro Protein Digestibility-Corrected Amino Acid Score = AAS × IVPD.

Table 21 – Amino acid composition, Amino Acid Score for adults, and IVPDCAAS of the raw and processed sesame seed meals.

AA composition (g/100g protein)	Requirement pattern[1] (g/100g protein)	Raw Sample		Cooking		Microwave		Ultrasound	
		AA	AAS[2] (%)	AA	AAS[2] (%)	AA	AAS[2] (%)	AA	AAS[2] (%)
Essential (EAA)									
Histidine (His)	1.5	3.46 ± 0.01^{C}	230.9 ± 0.1^{d}	2.65 ± 0.01^{A}	176.3 ± 0.1^{a}	2.71 ± 0.01^{B}	180.4 ± 0.1^{c}	2.65 ± 0.01^{A}	176.9 ± 0.5^{b}
Isoleucine (Ile)	3.0	5.92 ± 0.01^{C}	197.2 ± 0.2^{c}	4.30 ± 0.03^{A}	143.4 ± 1.0^{a}	4.43 ± 0.10^{B}	147.5 ± 3.2^{b}	4.25 ± 0.04^{A}	141.6 ± 1.4^{a}
Leucine (Leu)	5.9	9.04 ± 0.02^{C}	153.2 ± 0.3^{c}	6.33 ± 0.02^{A}	107.4 ± 0.3^{a}	6.42 ± 0.01^{B}	108.9 ± 0.2^{b}	6.37 ± 0.04^{A}	108.0 ± 0.6^{a}
Lysine (Lys)	4.5	3.59 ± 0.01^{C}	79.7 ± 0.3^{c}	2.62 ± 0.01^{B}	58.2 ± 0.2^{b}	2.62 ± 0.03^{B}	58.1 ± 0.6^{b}	2.45 ± 0.03^{A}	54.4 ± 0.7^{a}
Threonine (Thr)	2.3	5.04 ± 0.07^{C}	219.0 ± 3.1^{c}	3.79 ± 0.08^{B}	164.8 ± 3.5^{b}	3.68 ± 0.01^{A}	159.9 ± 0.1^{a}	3.75 ± 0.01^{AB}	163.1 ± 0.6^{ab}
Tryptophan (Trp)	0.6	1.23 ± 0.03^{C}	204.5 ± 6.0^{c}	1.25 ± 0.02^{C}	208.4 ± 3.9^{c}	1.06 ± 0.03^{B}	176.4 ± 4.7^{b}	0.83 ± 0.03^{A}	137.6 ± 1.5^{a}
Valine (Val)	3.9	6.91 ± 0.02^{D}	177.2 ± 0.4^{d}	4.81 ± 0.01^{B}	123.3 ± 0.3^{b}	4.91 ± 0.01^{C}	125.8 ± 0.3^{c}	4.75 ± 0.01^{A}	121.8 ± 0.4^{a}
Total sulfur amino acids (Met + Cys)	2.2	3.37 ± 0.03^{D}	153.3 ± 1.6^{d}	3.23 ± 0.02^{B}	146.9 ± 0.8^{b}	3.30 ± 0.03^{C}	149.8 ± 1.4^{c}	3.09 ± 0.01^{A}	140.4 ± 0.4^{a}
Total aromatic amino acids (Phe + Tyr)	3.8	11.84 ± 0.01^{D}	311.7 ± 0.1^{d}	8.52 ± 0.01^{B}	224.3 ± 0.3^{b}	8.57 ± 0.01^{C}	225.6 ± 0.3^{c}	8.45 ± 0.04^{A}	222.5 ± 1.1^{a}
Non-essential (NEAA)									
Alanine (Ala)	-	6.38 ± 0.03^{C}	-	4.54 ± 0.02^{A}	-	4.57 ± 0.01^{A}	-	4.66 ± 0.02^{B}	-
Arginine (Arg)	-	20.25 ± 0.17^{C}	-	15.39 ± 0.05^{AB}	-	15.26 ± 0.08^{A}	-	15.56 ± 0.01^{B}	-
Aspartic acid (Asp)	-	9.54 ± 0.02^{B}	-	7.88 ± 0.04^{A}	-	7.85 ± 0.01^{A}	-	7.90 ± 0.03^{A}	-
Glutamic acid (Glu)	-	23.61 ± 0.12^{C}	-	20.74 ± 0.02^{A}	-	20.70 ± 0.03^{A}	-	21.03 ± 0.01^{B}	-
Glycine (Gly)	-	7.09 ± 0.01^{C}	-	5.16 ± 0.03^{A}	-	5.15 ± 0.03^{A}	-	5.24 ± 0.01^{B}	-
Proline (Pro)	-	4.86 ± 0.02^{D}	-	3.92 ± 0.02^{A}	-	3.99 ± 0.04^{B}	-	4.10 ± 0.03^{C}	-
Serine (Ser)	-	6.46 ± 0.01^{D}	-	4.86 ± 0.02^{B}	-	4.80 ± 0.02^{A}	-	4.93 ± 0.04^{C}	-
Total EAA (g/100g protein)	-	39.27 ± 0.13^{C}	-	37.51 ± 0.04^{B}	-	37.68 ± 0.22^{B}	-	36.59 ± 0.03^{A}	-
Total NEAA (g/100g protein)	-	60.73 ± 0.13^{A}	-	62.49 ± 0.04^{B}	-	62.32 ± 0.22^{B}	-	63.41 ± 0.03^{C}	-
Total AA (g/100g sample)	-	36.86 ± 0.08^{D}	-	35.79 ± 0.07^{C}	-	32.42 ± 0.04^{A}	-	34.53 ± 0.04^{B}	-
First limiting amino acid	-	-	Lys	-	Lys	-	Lys	-	Lys
IVPDCAAS (%)	-	-	70.9	-	55.4	-	54.9	-	51.5

All values are means ± standard deviation. Processes were performed at 87.8 ºC, pH 8.0, and 37 min.

[1] WHO/FAO/UNU (2007) Expert Consultation Report for adults.

[2] AAS: Amino Acid Score = (mg of amino acid in 1 g of test protein/mg of amino acid in requirement pattern)×100.

[A–D] Different letters in the same row indicate a significant difference in the AA composition between samples for each amino acid ($p < 0.05$ by Tukey's test).

[a–d] Different letters in the same row indicate a significant difference in the AAS between samples for each essential amino acid ($p < 0.05$ by Tukey's test).

IVPDCAAS: In Vitro Protein Digestibility-Corrected Amino Acid Score = AAS × IVPD.

The FSM raw sample results (Table 20) also presented statistical differences ($p < 0.05$) between all processing treatments for each amino acid evaluated, except tryptophan (for microwave and ultrasound) and threonine. Essential amino acids (EAA) concentration decreased 2.0%, 1.7%, and 2.4% for cooking, microwave, and ultrasound, respectively, while total AA content was reduced 5.7%, 12.1%, and 5.5% compared to the raw sample. Additionally, regarding all processing, the results for FSM showed that the major reductions in amino acid concentration were isoleucine (~25%), valine (~16%), and arginine (~14%).

The SSM raw sample presented statistical differences ($p < 0.05$) between all processing treatments for each amino acid evaluated, except tryptophan (for cooking). Essential amino acids (EAA) and total AA content were reduced for cooking (5% and 3%, respectively), microwave (4% and 12%), and ultrasound (7% and 6%) when compared to the raw sample. The results showed that the major reductions in amino acid concentration regarding all processing were valine (~30%), leucine (~29%), aromatic amino acids (Phe + Tyr, ~28%), isoleucine (~28%), lysine (~28%), and alanine (~28%).

Studies showed that thermal treatments – such as cooking (100-120 ºC, 50-90 min) and microwave (15 min) – can decrease the amino acid concentration for chickpea, regarding lysine, tryptophan, arginine, total aromatic and sulfur-containing amino acids (CLEMENTE et al., 1998; ALAJAJI; EL-ADAWY, 2006). Although all processing techniques showed a slight decrease in amino acid concentration, the results for the total essential amino acids (EAA) and non-essential amino acids (NEAA) are excellent for these alternative protein sources. This behavior was also observed when verifying the Amino Acid Score (Table 19 – 21). Following the requirement pattern of EAA (WHO/FAO/UNU EXPERT CONSULTATION, 2007), the only limiting amino acid for all oilseed samples was lysine. These are very promising results as they demonstrate that even with reducing the amino acid composition when submitting the samples in cooking, microwave, and ultrasound processing, this decrease was not significant for reducing the amino acid score, except for lysine in SSM processed samples. Some interventions can be made to guarantee the proper lysine consumption, according to the requirement pattern: a) increasing the daily intake of this protein source (~125g of SSM raw sample); and b) supplementing the diet with plant proteins that are rich in lysine, such as chickpeas, soybeans, and peas, as demonstrated by Sá et al. (2020).

Concerning the in vitro protein digestibility-correct amino acid score (IVPDCAAS), the results for raw and processed PSM samples (Table 19), regarding lysine as the limiting amino acid, showed that processing was able to maintain the amino acid score for this source,

and increase the IVPDCAAS 7.2%, 6.1%, and 4.3%, for cooking (82.1%), microwave (81.3%), and ultrasound (79.9%), respectively, compared to the raw sample (76.6%). For FSM samples (Table 20), IVPDCAAS was increased by 10.8%, 8.6%, and 8.0% for cooking (85.0%), microwave (83.3%), and ultrasound (82.8%), respectively, compared to the raw sample (76.7%).

However, processing was not beneficial to increase the IVPDCAAS of SSM samples due to the decrease by 21.9%, 22.6%, and 27.4% of cooking (55.4%), microwave (54.9%), and ultrasound (51.5%) when compared to the SSM raw sample (70.9%) (Table 21). These findings agree with Nosworthy et al. (2018), reporting IVPDCAAS for cooked red (53.4%) and green lentils (51.4%). The processes reduced the lysine concentration and directly impacted the in vitro protein digestibility-correct amino acid score of sesame seed meal. Thus, if only considering the IVPDCAAS, all studied processing can be unnecessary interventions to increase the protein quality of sesame seed meal. However, concerning other important properties for food applications in the industry, these processes can increase some protein functionalities, as discussed in Section 5.2.5.

5.2.4 Antinutritional factors (ANFs)

As mentioned before, the presence of compounds considered antinutritional factors in food by-products from plant origin are unfavorable for protein digestion and they must be removed to increase protein digestibility (SÁ et al., 2021). The ANFs concentrations, regarding trypsin inhibition activity, tannin, and phytic acid, are shown in Table 22.

All oilseed by-products did not present tannins in the analysis performed. The raw PSM, FSM, and SSM showed trypsin inhibitor activity (33.1, 45.5, and 45.9 TIU/mg) in the same order of magnitude of traditional plant protein sources (e.g., soybean: 41.5 TIU/mg) (SAMARANAYAKA, 2017). Trypsin inhibitors are usually heat-stable and can require a long processing time for their inactivation (VAGADIA et al., 2018). The PSM samples processed by cooking, microwave, and ultrasound (87.8 ºC, pH 8.0, and 37 min) have trypsin inhibitor activity efficiently reduced by 77.3%, 84.0%, and 47.4%, respectively. For FSM, cooking, microwave, and ultrasound decreased the TIA by 24.8%, 49.7%, and 50.1%, while for SSM samples, processing was able to reduce 47%, 53%, and 55% of this ANF.

Table 22 – Antinutritional factors concentration of pumpkin seed, flaxseed, and sesame seed meals.

Antinutritional factors (ANFs)	Raw Sample	Cooking	Microwave	Ultrasound
PSM				
TIA (TIU/mg sample)	33.1 ± 0.5^{D}	7.5 ± 0.7^{B}	5.3 ± 0.2^{A}	17.4 ± 0.6^{C}
Phytic acid (µg/g sample)	35.0 ± 0.8^{D}	22.9 ± 0.5^{B}	33.4 ± 0.2^{C}	10.8 ± 0.1^{A}
Tannins (mg catechin/g sample)	n.d.^{A}	n.d.^{A}	n.d.^{A}	n.d.^{A}
FSM				
TIA (TIU/mg sample)	45.5 ± 0.8^{C}	34.2 ± 0.8^{B}	22.9 ± 0.6^{A}	22.7 ± 0.7^{A}
Phytic acid (µg/g sample)	22.0 ± 0.1^{C}	n.d.^{A}	n.d.^{A}	2.0 ± 0.2^{B}
Tannins (mg catechin/g sample)	n.d.^{A}	n.d.^{A}	n.d.^{A}	n.d.^{A}
SSM				
TIA (TIU/mg sample)	45.9 ± 0.9^{C}	20.8 ± 0.8^{A}	21.5 ± 0.6^{A}	24.5 ± 0.6^{B}
Phytic acid (µg/g sample)	26.1 ± 0.2^{D}	7.5 ± 0.3^{B}	17.3 ± 0.4^{C}	4.9 ± 0.7^{A}
Tannins (mg catechin/g sample)	n.d.^{A}	n.d.^{A}	n.d.^{A}	n.d.^{A}

All values are means ± standard deviation.
Processes were performed at 87.8 ºC, pH 8.0, and 37 min of processing time.
FSM = flaxseed meal; n.d.: not detected; PSM = pumpkin seed meal; SSM: sesame seed meal. TIA: trypsin inhibitory activity. TIU: trypsin inhibitory unit;
$^{A–C}$ Different letters in the same row indicate a significant difference between samples for each analysis ($p < 0.05$ by Tukey's test).

The highest level of phytic acid was noticed in the raw pumpkin seed meal sample (0.0035 g/100 g), which is extremely lower than traditional sources of plant proteins (e.g., pea: 1.2; soybean: 2.0; chickpea: 1.5; common bean: 1.6; and rice: 0.7 g/100 g) (SÁ et al., 2021). The low phytic acid content may be an oil extraction consequence that changes the chemical composition due to chemical affinity, highlighting the importance of the oil extraction step on improving protein digestion by reducing this ANF. However, cooking, microwave, and ultrasound treatments further reduced phytic acid levels for pumpkin seed meals by 34.6%, 4.6%, and 69.1%, respectively; and for sesame seed meals by 81%, 34%, and 71%. Furthermore, cooking and microwave were able to total inactivate phytic acid for flaxseed meals, while ultrasound reduced this ANF by 90.9%. The decrease in the ANFs concentration performed by thermal processing and ultrasound is compatible with the increase in IVPD results presented by the experimental design (Table 12 – 14).

Few studies evaluated the ANFs concentration of oilseed by-products and plant proteins when using emerging technologies, such as ultrasound. Besides, thermal processing

and ultrasound influence on the ANFs concentration of pumpkin seed, flaxseed, and sesame seed meals are also scarce. Therefore, the antinutritional factors evaluated in terms of the content of phytic acid, tannins, and trypsin inhibitor activity indicated raw and processed PSM, FSM, and SSM samples as promising protein sources for humans.

5.2.5 Functional properties

Protein functionality has critical importance in defining the applicability of plant proteins flours, concentrates, and isolates, which affect the physicochemical characteristics of food products (texture, appearance, stability, cohesion-adhesion, elasticity, and viscosity). Intrinsic and extrinsic factors (e.g., protein structure, amino acid composition, hydrophobicity, medium pH, salts, temperature, pressure, and ionic strength) can influence the functional properties of protein-containing foods (GENÇDAĞ; GÖRGÜÇ; YILMAZ, 2020). Protein extraction and processing may change those functional properties; thus, studying the process parameters is essential to understand the impact on food products' functional and physicochemical properties.

It is worth mentioning that all oilseed meals samples used to conduct the protein techno-functional properties assays were not seed protein isolates. These analyses were conducted to obtain responses regarding the functionalities behaviors of the protein inserted in the plant matrix. Other compounds present in the matrix (lipids, carbohydrates, fibers, and others) can greatly influence these functional results. All the processed samples were performed at 87.8 ºC, pH 8.0, and 37 min of processing time due to the best results of IVPD.

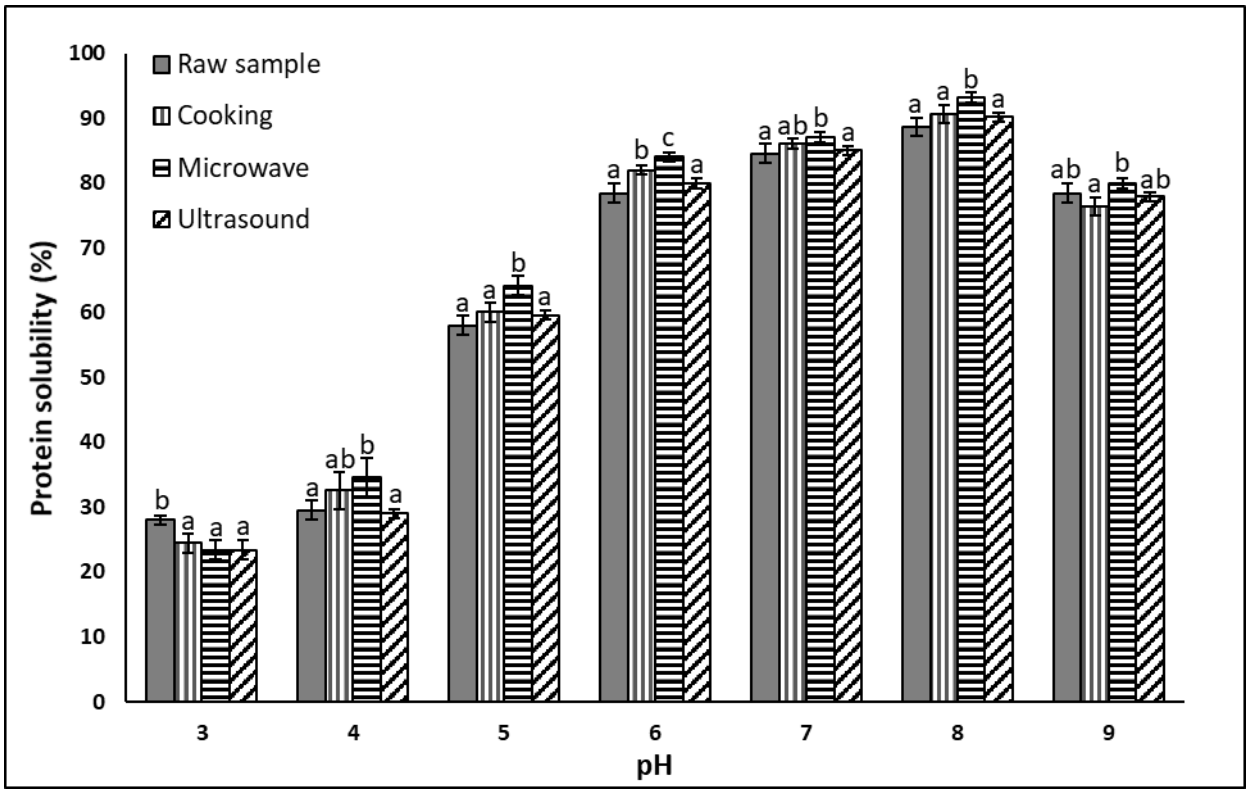
5.2.5.1 Protein solubility in the plant matrix

The protein solubility is highly influenced by hydrophilicity/hydrophobicity balance, which depends on the amino acid composition, particularly at the protein surface. Higher solubility is related to the presence of a low number of hydrophobic residues, elevated net charge and the electrostatic repulsion and ionic hydration occurring at pH above and below the isoelectric pH (pI) (ONSAARD, 2012).

PS of PSM, FSM, and SSM samples as a function of pH is shown in Figure 6 – 8. Similar results have been described previously in the literature for pumpkin seed (LAZOS, 1992; MANSOUR et al., 1993b; REZIG et al., 2013), flaxseeds (KRAUSE; SCHULTZ;

DUDEK, 2002; MARTÍNEZ-FLORES et al., 2006; LAN et al., 2020), and sesame seeds (KHALID; BABIKER; EL TINAY, 2003; CAPELLINI et al., 2019), but the solubility profile of these oilseed proteins are remarkably different in various salt solutions.

Figure 6 – Effect of pH on protein solubility in plant matrix of pumpkin seed meals.



Processes were performed at 87.8 ºC, pH 8.0, and 37 min of processing time.
[a–c] Different letters in the columns for each pH value indicate a significant difference between the raw and processed samples ($p < 0.05$ by Tukey's test).

The solubility of protein is very low near the isoelectric point (pI). The pI for pumpkin seed, flaxseed, and sesame seed is near 3.81 – 5.39; 4.25; and 4.50, respectively (LAZOS, 1992; ZHAO et al., 2012; HELLEBOIS et al., 2021), which corroborates with the results presented here. In this case, the protein inside the seed matrix went to the supernatant, and the protein solubility was measured of this amount presented in the aqueous medium. Regarding the PSM samples, the minimum protein solubility was 23.4% at pH 3 for microwave and ultrasound samples. However, the solubilization was highly improved at alkaline pH value (8), up to 93%, for raw and processed samples. Similar results were found in FSM samples. 44.3% of protein solubility in plant matrix for the raw sample in pH 4 was the minimum observed, but the solubilization was extremely improved at alkaline pH values (8 – 9), up to 98%, for raw and processed samples.

Figure 7 – Effect of pH on protein solubility in plant matrix of flaxseed meals.

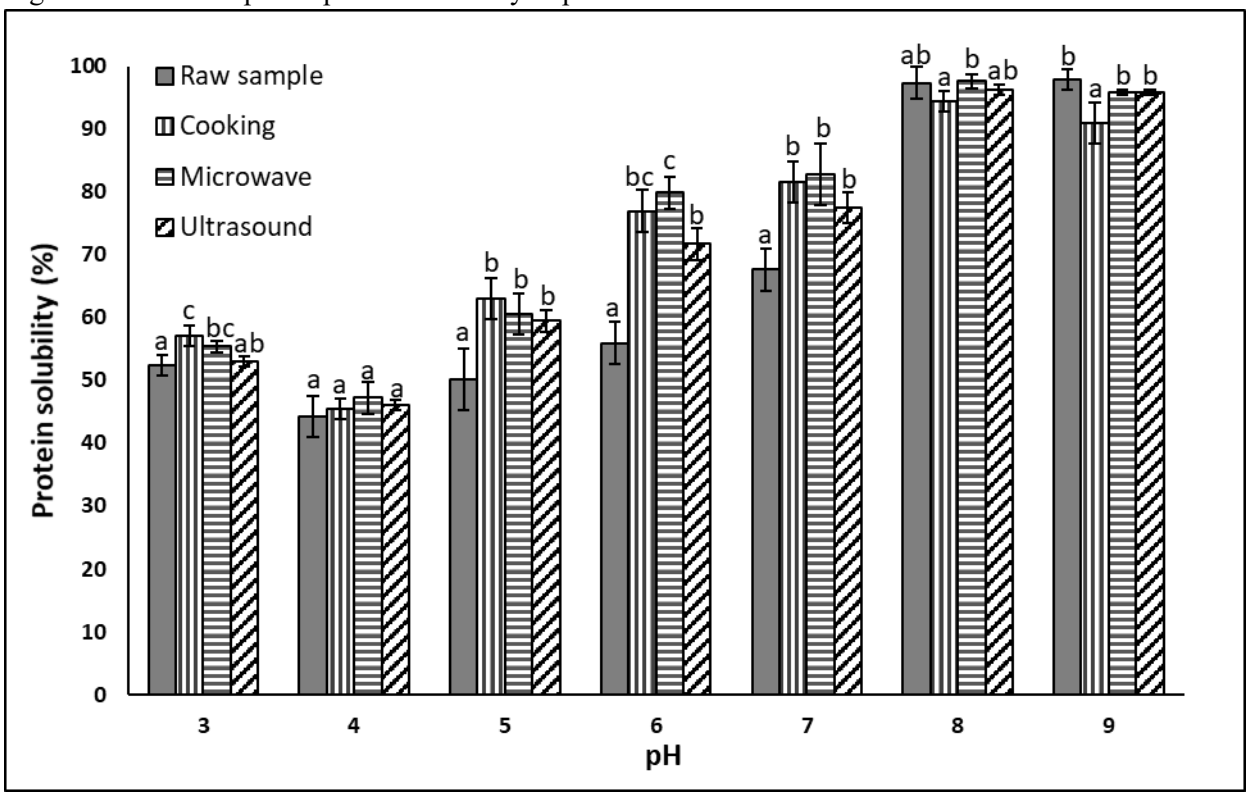
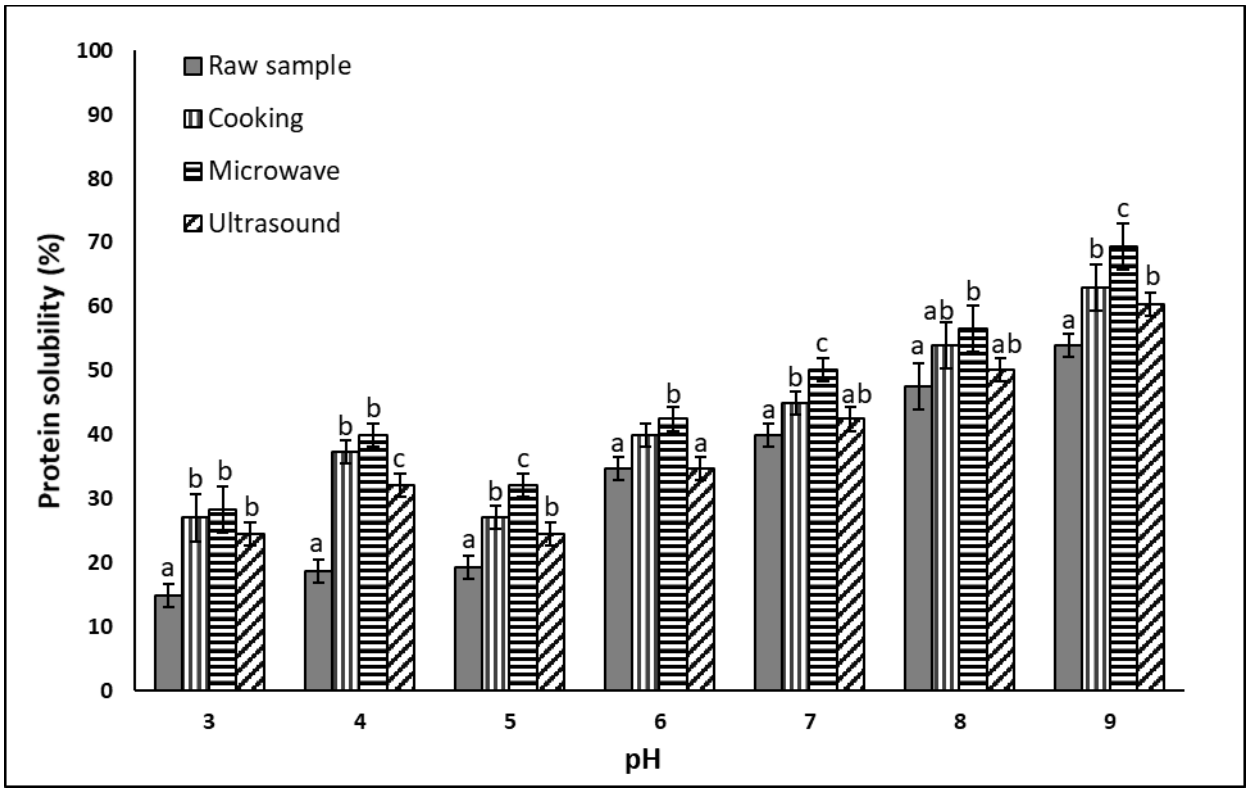


Figure 8 – Effect of pH on protein solubility in plant matrix of sesame seed meals.



Processes were performed at 87.8 ºC, pH 8.0, and 37 min of processing time.

[a–c] Different letters in the columns for each pH value indicate a significant difference between the raw and processed samples ($p < 0.05$ by Tukey's test).

Besides, although the minimum protein solubility was 15% for the raw SSM sample at pH 3, higher protein solubilization was observed at alkaline pH values (8 – 9), up to 70%, for SSM raw and processed samples. Furthermore, other compounds present in the matrix, such as lipids, carbohydrates, and fibers, may influence the protein solubility results.

High protein solubility is important for food formulation. Usually, the utilization of plant proteins as ingredients for the food industry is limited due to their extremely low solubility at neutral pH, except for the soybean, pea, canola (CONTRERAS et al., 2019), and cowpea (PEYRANO et al., 2021). However, the protein solubility in the plant matrix of the processed PSM, FSM, and SSM samples presented here demonstrated promising results for applications in the food industry. Furthermore, the high solubility of PSM, FSM, SSM samples at pH 8 may have also influenced the increase in IVPD when the processing treatments occurred at this pH value, as discussed in Section 5.2.2, due to these oilseed proteins being soluble and more available in the reaction medium for the digestive enzymes attack, simulated in the in vitro digestibility analysis.

5.2.5.2 Foaming capacity and stability in the plant matrix

Protein foaming agents should stabilize foams rapidly and effectively at low concentrations and perform as an effective foaming agent over the pH range and in the medium with foam inhibitors (e.g., fat, alcohol, or flavor substances) (ZAYAS, 1997). FC and FS of raw and processed PSM, FSM, and SSM samples are presented in Table 23. The foaming properties of PSM, FSM, and SSM raw samples were analyzed at their original pH (6.33, 5.91, and 6.90, respectively). For processed samples, the properties were evaluated at pH 8.00, which was the best processing condition found for IVPD.

Although the PSM raw sample presented a foaming capacity of 75.6%, cooking, microwave, and ultrasound increased FC by 9.8%, 6.6%, and 4.4%, respectively, with results of FC up to 83%. This result was superior when compared to those described previously by El-Adawy and Taha (2001) (18.7%) and by Giami and Isichei (1999) (18.5%) for pumpkin seed flours. The FSM raw sample presented an inferior foaming capacity of 9.5%, but cooking, microwave, and ultrasound increased FC up to 37%. This result was comparable to those described previously by Martínez-Flores et al. (2006) (12 – 42%) for flaxseed protein concentrates, depending on pH values.

Table 23 – Functional properties of pumpkin seed, flaxseed, and sesame seed meals.

Functional properties	Raw Sample	Cooking	Microwave	Ultrasound
PSM				
FC (%)	75.6 ± 0.8^{A}	83.0 ± 0.7^{D}	80.6 ± 0.9^{C}	78.9 ± 0.9^{B}
FS (%)	19.5 ± 0.7^{B}	23.0 ± 0.7^{A}	23.5 ± 0.7^{A}	23.1 ± 0.9^{A}
WHC (g/g)	1.55 ± 0.02^{C}	1.89 ± 0.03^{AB}	1.94 ± 0.04^{B}	1.87 ± 0.05^{A}
OHC (g/g)	0.78 ± 0.01^{B}	0.90 ± 0.05^{A}	0.86 ± 0.01^{A}	0.84 ± 0.02^{A}
FSM				
FC (%)	9.5 ± 0.7^{B}	34.5 ± 0.7^{A}	36.9 ± 0.9^{C}	35.0 ± 0.7^{A}
FS (%)	5.8 ± 0.4^{B}	10.3 ± 0.4^{A}	10.9 ± 0.2^{C}	9.8 ± 0.4^{A}
WHC (g/g)	2.90 ± 0.01^{A}	3.00 ± 0.08^{BC}	3.07 ± 0.03^{C}	2.97 ± 0.01^{AB}
OHC (g/g)	0.87 ± 0.01^{B}	0.96 ± 0.01^{A}	0.98 ± 0.02^{A}	0.95 ± 0.02^{A}
SSM				
FC (%)	51.9 ± 0.6^{A}	96.9 ± 0.9^{C}	94.8 ± 0.4^{B}	94.3 ± 0.4^{B}
FS (%)	15.3 ± 0.4^{A}	49.4 ± 0.9^{D}	45.6 ± 0.9^{C}	40.3 ± 0.4^{B}
WHC (g/g)	1.73 ± 0.08^{A}	2.01 ± 0.01^{B}	2.06 ± 0.01^{B}	1.99 ± 0.02^{B}
OHC (g/g)	0.93 ± 0.04^{A}	1.03 ± 0.01^{B}	1.10 ± 0.01^{C}	1.06 ± 0.02^{BC}

All values are means ± standard deviation.
Processes were performed at 87.8 ºC, pH 8.0, and 37 min of processing time.
FC: foaming capacity; FS: foaming stability; n.d.: not detected; OHC: oil-holding capacity; TIA: trypsin inhibitory activity. TIU: trypsin inhibitory unit; WHC: water-holding capacity.
$^{A–C}$ Different letters in the same row indicate a significant difference between samples for each analysis ($p < 0.05$ by Tukey's test).

Also, the SSM raw sample presented 51.9% of foaming capacity. Cooking, microwave, and ultrasound improved FC by 87%, 83%, and 82%, respectively, with results of FC up to 97% for SSM samples. A similar result (100%) has been described previously by Khalid et al. (2003) for sesame seed protein isolate at the same pH conditions. However, the samples evaluated in this study were not seed protein isolates and other compounds present in the matrix (lipids, carbohydrates, fibers) may influence the foaming results.

These foaming behaviors were likely due to the increased net charges on the protein, weakening the hydrophobic interactions, increasing the protein flexibility, and allowing the protein to diffuse more rapidly to the air-water interface to encapsulate air particles, which enhances the foam formation (KHALID; BABIKER; EL TINAY, 2003). The best protein foaming agents in the food industry are generally egg white, gelatins, casein, soybean proteins, and gluten (ZAYAS, 1997). These good FC results for PSM and SSM samples demonstrated

viability for their applications in the food industry due to plant proteins with foaming properties being good for salad dressings and soups (ONSAARD, 2012).

The raw PSM sample had foaming stability of 19.5%, while samples processed by cooking, microwave, and ultrasound increased PSM FS up to 24%. For FSM, the results of FS were inferior, 5.8% for the raw sample and up to 11% for the processed samples. Also, the foaming stability was 15% for the raw sesame seed meal, while cooking, microwave, and ultrasound increased FS by 223%, 198%, and 163%, respectively, with results up to 50%.

Although all oilseed samples foaming properties decreased after 30 min of analysis, some interventions can assure higher foaming stability. The addition of salts can significantly enhance protein FS due to increased solubility and surface activity of the soluble protein (KHALID; BABIKER; EL TINAY, 2003).

5.2.5.3 Water- and oil-holding capacities in the plant matrix

Water-holding capacity is mainly attributed to a protein matrix's ability (e.g., protein particles, gels, or muscle) to retain and absorb water against gravity, including bound, capillary, hydrodynamic, and physically entrapped water (ONSAARD, 2012). WHC is an important parameter in meat processing, affecting the product's juiciness, tenderness, and taste (MU; SUN; WANG, 2017). Oil-holding capacity refers to the oil physical entrapment, the number of nonpolar side chains on proteins, and their different conformational features that bind hydrocarbon chains on the fatty acids (KHALID; BABIKER; EL TINAY, 2003). Proteins with good oil-holding capacities can be widely used in egg yolk products, meat products, dairy products, coffee mate, dough, and cake pastes (MU; SUN; WANG, 2017). WHC and OHC of raw and processed PSM, FSM, and SSM samples are presented in Table 23.

The WHC for raw PSM was 1.55 g H_2O/g, while samples processed by cooking, microwave, and ultrasound increased WHC 22%, 25%, and 21%, respectively, with results up to 1.95 g H_2O/g. A similar result (1.99 g H_2O/g) was found by Lovatto et al. (2020) for pumpkin seed meal. For raw FSM, WHC was elevated (2.90 g H_2O/g), and cooking, microwave, and ultrasound were able to further increase WHC up to 3.10 g H_2O/g. Besides, the water-holding capacity was 1.7 g H_2O/g for the raw sesame seed meal, while SSM processed by cooking, microwave, and ultrasound increased 16%, 19%, and 15% of WHC, respectively, with results up to 2.1 g H_2O/g. The same value (2.1 g H_2O/g) was found by Khalid et al. (2003) for sesame seed protein isolate, within the range of protein concentrates commercial values (1.9 – 2.2).

They suggested that carbohydrates and other components of the plant matrix may impair WHC, while protein isolates have great ability to swell, dissociate and unfold, exposing additional binding sites, increasing their WHC. However, for this functionality, WHC results for processed PSM, FSM, and SSM samples showed promising viability for applications in the food industry, similar to oilseed concentrates and isolates.

The OHC for raw PSM was 0.78 g oil/g, while samples processed by cooking, microwave, and ultrasound increased up to 0.90 g oil/g. A similar result (0.72 g oil/g) was found by Lovatto et al. (2020) for phosphorylated protein concentrate from pumpkin seed meal. For raw FSM, OHC was 0.87 g oil/g, and cooking, microwave, and ultrasound were able to further increase WHC up to 1.00 g oil/g. Besides, the oil-holding capacity was 0.9 g H_2O/g for the raw sesame seed meal, while SSM processed by cooking, microwave, and ultrasound increased OHC by 11%, 18%, and 14%, respectively, with results up to 1.1 g oil/g, which was lower to results presented by previously by Khalid et al. (2003) for sesame seed protein isolate and soybean.

Other compounds present in the matrix, such as lipids, carbohydrates, and fibers, may influence the WHC and OHC results; however, for the PSM, FSM, and SSM samples, those demonstrated viability for their applications in the food industry due to plant proteins with high oil- and water-holding capacities being desirable for use in meat (ONSAARD, 2012) or meat analogs products.

5.3 FINAL CONSIDERATIONS OF THE CHAPTER

Cooking, microwave, and ultrasound at different conditions of temperature, pH, and time were processing techniques that impacted the nutritional quality and functional properties of pumpkin seed, flaxseed, and sesame seed meals. The surface responses showed that IVPD can greatly depend on the temperature, time, and pH for cooking, microwave, and ultrasound processing. Processing at 87.8 ºC, pH 8.0, and 37 min increased the IVPD response regarding the oilseed samples, from 83.7% up to 96.1%, which is a high value for a plant protein source. Similarly, the ANFs have reduced: TIA declined up to 84%, and phytic acid was able to be completely inactivated, while tannins were not detected. Thus, these techniques can be used as potential methods of processing oilseed by-products to increase protein digestibility and eliminate antinutritional factors.

Lysine was the first limiting amino acid for all oilseed meal samples. Although cooking, microwave, and ultrasound influenced the amino acid profile, the processing did not decrease the amino score for the essential amino acids, except for lysine in SSM, which directly affected the processed samples IVPDCAAS. Concerning techno-functional properties, the protein solubility, water- and oil-holding capacity, and foaming properties in plant matrix demonstrated that processed oilseed meals are promising and can be used as alternative protein sources aiming food formulation systems.

Regarding the influence of cooking, microwave, and ultrasound on the nutritional and functional quality, one can conclude that oilseed by-products from the oil extraction industries are sustainable and high-quality protein sources. These sustainable alternative sources can be used as technological ingredients for food formulations and may become extra income for the industry while minimizing large waste disposals.

CHAPTER VI

6 CONCLUSIONS

Nowadays, food security and the demand for high-quality foods aiming to meet human nutritional needs while promoting health is a constant challenge, primarily in a scenario of the increasing world population and constrained environmental resources. Plant protein digestibility and amino acid bioavailability are critical aspects when looking for diversification of protein sources. For this purpose, this thesis presented a valuable and promising alternative for producing proteins from agro-industrial residues regarding their nutritional composition and protein quality. The results revealed that it is possible to obtain high nutritional value proteins from these residues to be an excellent alternative of protein source for human consumption.

A ranking among the screening of potential sources was determined: chia seed meal > brown flaxseed meal > sesame seed meal > pumpkin seed meal > flaxseed meal > grapeseed meal > grapeseed meal flour. Furthermore, the utilization of blends of these raw by-products is very interesting precisely for overcoming the deficiencies pointed out in the profile of essential amino acids in the sources. This work suggests two potential blends, such as brown flaxseed meal (lysine deficiency) with pumpkin seed meal (sulfur amino acid deficiency) and sesame seed meal (lysine deficiency) with pumpkin seed meal (sulfur amino acid deficiency).

Pumpkin seed, flaxseed, and sesame seed meals were selected to be processed by cooking, microwave, and ultrasound. The best parameters were temperature 87.8 ºC, pH 8.0, and 37 min of processing time, which increased IVPD responses for pumpkin seed, flaxseed, and sesame seed meals up to 96.1%. Regarding the antinutritional factors concentration, TIA declined up to 84%, and phytic acid was completely inactivated, while tannins were not detected in the samples. Therefore, cooking, microwave, and ultrasound are potential processing methods to increase protein digestibility and eliminate antinutritional factors of oilseed residues.

About the amino acid composition, lysine was the first limiting amino acid for all oilseed meal samples. Processing greatly influenced the amino acid composition by reducing some essential amino acids. However, cooking, microwave, and ultrasound did not decrease the amino score for the essential amino acids, except for lysine in sesame seed meals. They directly negatively affected the IVPDCAAS of processed sesame seed samples. Concerning techno-functional properties, the protein solubility, water- and oil-holding capacity, and

foaming properties in plant matrix demonstrated that processed oilseed meals are promising and can be used as alternative protein sources aiming food formulation systems.

Although few studies reported the impact of thermal processing on plant proteins' amino acid profile, insufficient studies evaluate the amino acid composition of plant proteins when using emerging technologies, such as ultrasound. Information about the nutrition quality of pumpkin seed, flaxseed, and sesame seed meals is also scarce. Besides, no data is presented in the literature concerning the thermal processing and ultrasound influence on the protein quality of these sustainable sources, which brings the novelty of this thesis.

There is a constant requirement for protein quality and amino acid availability, which are critical aspects of meeting human nutritional needs, which are scientific and technological challenges. Based on the results exposed here, using these oilseed by-products for protein production could provide extra income and simultaneously help minimize waste disposal problems. In addition, the use of oilseed meals as possible ingredients for food formulations is very interesting, due to the presence of starches, lecithin, and lipids of high nutritional quality, in addition to the high protein content. Finally, these sources are promising, as they increase the spectrum choice of plant proteins from renewable sources. Therefore, including these oilseed by-products is very recommended for healthy diets and industrial applications, further expanding the range of nutritious ingredients available for human consumption. These alternative sources can be a golden opportunity for protein utilization as supplements for athletes, older people with nutritional deficiency, and malnourished children.

6.1 FURTHER WORK SUGGESTIONS

- Perform emerging technologies, such as high pressure, cold plasma, and enzymatic processes to evaluate their impact on the protein quality and functional properties of plant proteins and oilseed by-products;
- Evaluate the emulsifying and gelling properties of the oilseed meals;
- Assess postprandial aminoacidemia (i.e., the variation in plasma amino acid concentration) in healthy humans after ingesting the selected protein samples from oilseed by-products compared to a standard protein (casein or whey), aiming to evaluate the amino acid bioavailability in humans.

REFERENCES

ABU-TARBOUSH, H. M.; AHMED, S. A. B. Studies on karkade (Hibiscus sabdariffa): Protease inhibitors, phytate, in vitro protein digestibility and gossypol content. **Food Chemistry**, v. 56, n. 1, p. 15–19, 1996.

ADENEKAN, M. K. et al. Effect of isolation techniques on the characteristics of pigeon pea (Cajanus cajan) protein isolates. **Food Science and Nutrition**, v. 6, n. 1, p. 146–152, 2018.

AGUILAR, E. G. et al. Evaluation of the nutritional quality of the grain protein of new amaranths varieties. **Plant Foods for Human Nutrition**, v. 70, n. 1, p. 21–26, 2015.

AIDER, M.; BARBANA, C. Canola proteins: composition, extraction, functional properties, bioactivity, applications as a food ingredient and allergenicity - A practical and critical review. **Trends in Food Science & Technology**, v. 22, n. 1, p. 21–39, 2011. Disponível em: <http://dx.doi.org/10.1016/j.tifs.2010.11.002>.

AKESON, W. R.; STAHMANN, M. A. A pepsin pancreatin digest index of protein quality evaluation. **The Journal of Nutrition**, v. 83, p. 257–261, 1964.

AL-RUWAIH, N. et al. High-pressure assisted enzymatic proteolysis of kidney beans protein isolates and characterization of hydrolysates by functional, structural, rheological and antioxidant properties. **LWT - Food Science and Technology**, v. 100, p. 231–236, 2019. Disponível em: <https://doi.org/10.1016/j.lwt.2018.10.074>.

AL JUHAIMI, F.; ÖZCAN, M. M. Effect of cold press and soxhlet extraction systems on fatty acid, tocopherol contents, and phenolic compounds of various grape seed oils. **Journal of Food Processing and Preservation**, v. 42, n. 1, 2018.

ALAJAJI, S. A.; EL-ADAWY, T. A. Nutritional composition of chickpea (Cicer arietinum L.) as affected by microwave cooking and other traditional cooking methods. **Journal of Food Composition and Analysis**, v. 19, n. 8, p. 806–812, 2006.

ALBARRACÍN, M.; JOSÉ GONZÁLEZ, R.; DRAGO, S. R. Soaking and extrusion effects on physicochemical parameters, phytic acid, nutrient content and mineral bio-accessibility of whole rice grain. **International Journal of Food Sciences and Nutrition**, v. 66, n. 2, p. 210–215, 2015.

ALEMAYEHU, F. R.; BENDEVIS, M. A.; JACOBSEN, S. E. The potential for utilizing the seed crop amaranth (Amaranthus spp.) in East Africa as an alternative crop to support food security and climate change mitigation. **Journal of Agronomy and Crop**

Science, v. 201, n. 5, p. 321–329, 2015.

ALETOR, O. Protein quality evaluation and in vitro multi-enzyme digestibility of some plant protein isolates and concentrates. **Archiva Zootechnica**, v. 15, n. 4, p. 5–16, 2012.

ALONSO, R.; AGUIRRE, A.; MARZO, F. Effects of extrusion and traditional processing methods on antinutrients and in vitro digestibility of protein and starch in faba and kidney beans. **Food Chemistry**, v. 68, n. 2, p. 159–165, 2000.

ALVAREZ-JUBETE, L.; ARENDT, E. K.; GALLAGHER, E. Nutritive value of pseudocereals and their increasing use as functional gluten-free ingredients. **Trends in Food Science and Technology**, v. 21, n. 2, p. 106–113, 2010. Disponível em: <http://dx.doi.org/10.1016/j.tifs.2009.10.014>.

AMAGLIANI, L. et al. The composition, extraction, functionality and applications of rice proteins: A review. **Trends in Food Science and Technology**, v. 64, p. 1–12, 2017. Disponível em: <http://dx.doi.org/10.1016/j.tifs.2017.01.008>.

ANAYA, K. et al. Growth impairment caused by raw linseed consumption: Can trypsin inhibitors be harmful for health? **Plant Foods for Human Nutrition**, v. 70, n. 3, p. 338–343, 2015.

ANDERSON, J. W. et al. Health benefits of dietary fiber. **Nutrition Reviews**, v. 67, n. 4, p. 188–205, 2009.

ANNOR, G. A. et al. Why do millets have slower starch and protein digestibility than other cereals? **Trends in Food Science and Technology**, v. 66, p. 73–83, 2017. Disponível em: <http://dx.doi.org/10.1016/j.tifs.2017.05.012>.

AOAC INTERNATIONAL. **Official methods of analysis of AOAC International**. 19th. ed. [s.l: s.n.]

ARRIBAS, C. et al. The impact of extrusion on the nutritional composition, dietary fiber and in vitro digestibility of gluten-free snacks based on rice, pea and carob flour blends. **Food and Function**, v. 8, n. 10, p. 3654–3663, 2017.

ARYA, S. S.; SALVE, A. R.; CHAUHAN, S. Peanuts as functional food: a review. **Journal of Food Science and Technology**, v. 53, n. 1, p. 31–41, 2016.

BARAC, M. B. et al. Comparative study of the functional properties of three legume seed isolates: adzuki, pea and soybean. **Journal of Food Science**, v. 52, n. 5, p. 2779–2787, 2015.

BARTKIENE, E.; JUODEIKIENE, G.; VIDMANTIENE, D. Nutritional quality of fermented defatted soya and flaxseed flours and their effect on texture and sensory

characteristics of wheat sourdough bread. **International Journal of Food Sciences and Nutrition**, v. 63, n. 6, p. 722–729, 2012.

BERNARDO, C. O. et al. Impact of extruded sorghum genotypes on the rehydration and sensory properties of soluble beverages and the Brazilian consumers ' perception of sorghum and cereal beverage using word association. **Journal of Cereal Science**, v. 89, n. March, p. 102793, 2019. Disponível em: <https://doi.org/10.1016/j.jcs.2019.102793>.

BERNO, L. I.; GUIMARÃES-LOPES, T. G.; CANNIATTI-BRAZACA, S. G. Avaliação da composição centesimal, digestibilidade e atividade inibitória de tripsina em produtos derivados de soja (Glycine max). **Alimentos e Nutricão**, v. 18, n. 3, p. 277–282, 2007.

BERRYMAN, C. E. et al. Protein intake trends and conformity with the Dietary Reference Intakes in the United States: analysis of the National Health and Nutrition Examination Survey, 2001–2014. **The American Journal of Clinical Nutrition**, v. 108, p. 1–9, 2018. Disponível em: <https://academic.oup.com/ajcn/advance-article/doi/10.1093/ajcn/nqy088/5042716>.

BESSADA, S. M. F.; BARREIRA, J. C. M.; OLIVEIRA, M. B. P. P. **Pulses and food security: Dietary protein, digestibility, bioactive and functional propertiesTrends in Food Science and Technology**, 2019. .

BHATTY, N.; GILANI, A. H.; NAGRA, S. A. Effect of cooking and supplementation on nutritional value of gram (Cicer arietinum). **Nutrition Research**, v. 20, n. 2, p. 297–307, 2000.

BILGIN, Ö.; TÜRKER, A.; TEKINAY, A. A. The use of Hazelnut meal as a substitute for soybean meal in the diets of rainbow trout (Oncorhynchus mykiss). **Turkish Journal of Veterinary and Animal Sciences**, v. 31, n. 3, p. 145–151, 2007.

BISHNOI, S.; KHETARPAUL, N. Protein digestibility of vegetables and field peas (Pisum sativum): Varietal differences and effect of domestic processing and cooking methods. **Plant Foods for Human Nutrition**, v. 46, p. 71–76, 1994.

BOLAND, M. J. et al. The future supply of animal-derived protein for human consumption. **Trends in Food Science & Technology**, v. 29, n. 1, p. 62–73, 2013. Disponível em: <http://dx.doi.org/10.1016/j.tifs.2012.07.002>.

BOYE, J.; WIJESINHA-BETTONI, R.; BURLINGAME, B. Protein quality evaluation twenty years after the introduction of the protein digestibility corrected amino acid score method. **British Journal of Nutrition**, v. 108, p. S183–S211, 2012.

BURNS, R. E. Method for estimation of tannin in grain sorghum. **Agronomy Journal**,

v. 63, p. 511–512, 1971.

BUYUKCAPAR, H. M.; KAMALAK, A. Partial replacement of fish and soyabean meal protein in mirror carp (Cyprinus carpio) diets by protein in hazelnut meal. **South African Journal of Animal Sciences**, v. 37, n. 1, p. 35–44, 2007.

BYANJU, B. et al. Effect of high-power sonication pretreatment on extraction and some physicochemical properties of proteins from chickpea, kidney bean, and soybean. **International Journal of Biological Macromolecules**, v. 145, p. 712–721, 2020. Disponível em: <https://doi.org/10.1016/j.ijbiomac.2019.12.118>.

ÇABUK, M. B. et al. Effect of fermentation on the protein digestibility and levels of non-nutritive compounds of pea protein concentrate. **Food Technology and Biotechnology**, v. 56, n. 2, p. 257–264, 2018.

CAMPBELL, L.; REMPEL, C. B.; WANASUNDARA, J. P. D. Canola/Rapessed protein: Future opportunities and directions - Workshop proceedings of IRC 2015. **Plants**, v. 5, n. 17, p. 1–7, 2016.

CANNIATTI-BRAZACA, S. G. Valor nutricional de produtos de ervilha em comparação com a ervilha fresca. **Ciencia E Tecnologia De Alimentos**, v. 26, n. 4, p. 766–771, 2006. Disponível em: <http://www.redalyc.org/articulo.oa?id=395940080009>.

CAPELLINI, M. C. et al. Alcoholic extraction of sesame seed cake oil: Influence of the process conditions on the physicochemical characteristics of the oil and defatted meal proteins. **Journal of Food Engineering**, v. 240, n. April 2018, p. 145–152, 2019. Disponível em: <https://doi.org/10.1016/j.jfoodeng.2018.07.029>.

CARVALHO, C. W. P. et al. Relative effect of particle size on the physical properties of corn meal extrudates: Effect of particle size on the extrusion of corn meal. **Journal of Food Engineering**, v. 98, n. 1, p. 103–109, 2010. Disponível em: <http://dx.doi.org/10.1016/j.jfoodeng.2009.12.015>.

CHAKRABORTY, P.; BHATTACHARYYA, D. K.; GHOSH, M. Extrusion treated meal concentrates of Brassica juncea as functionally improved ingredient in protein and fiber rich breadstick preparation. **LWT - Food Science and Technology**, v. 142, n. January, p. 111039, 2021. Disponível em: <https://doi.org/10.1016/j.lwt.2021.111039>.

CHEMAT, F.; HUMA, Z.; KHAN, M. K. Applications of ultrasound in food technology: Processing, preservation and extraction. **Ultrasonics - Sonochemistry**, v. 18, n. 4, p. 813–835, 2011. Disponível em: <http://dx.doi.org/10.1016/j.ultsonch.2010.11.023>.

CHEN, L. et al. Effects of ultrasound pretreatment on the enzymatic hydrolysis of soy

protein isolates and on the emulsifying properties of hydrolysates. **Journal of Agricultural and Food Chemistry**, v. 59, p. 2600–2609, 2011.

CHEW, P. G.; CASEY, A. J.; JOHNSON, S. K. Protein quality and physico-functionality of Australian sweet lupin (Lupinus angustifolius cv. Gungurru) protein concentrates prepared by isoelectric precipitation or ultrafiltration. **Food Chemistry**, v. 83, n. 4, p. 575–583, 2003.

CHIESA, S.; GNANSOUNOU, E. Protein extraction from biomass in a bioethanol refinery – Possible dietary applications: Use as animal feed and potential extension to human consumption. **Bioresource Technology**, v. 102, n. 2, p. 427–436, 2011. Disponível em: <http://dx.doi.org/10.1016/j.biortech.2010.07.125>.

CLEMENTE, A. et al. Effect of cooking on protein quality of chickpea (Cicer arietinum) seeds. **Food Chemistry**, v. 62, n. 1, p. 1–6, 1998.

CODA, R. et al. Improvement of the protein quality of wheat bread through faba bean sourdough addition. **LWT - Food Science and Technology**, v. 82, p. 296–302, 2017. Disponível em: <http://dx.doi.org/10.1016/j.lwt.2017.04.062>.

CONDE, J. M. et al. Effect of enzymatic treatment of extracted sunflower proteins on solubility, amino acid composition, and surface activity. **Journal of Agricultural and Food Chemistry**, v. 53, n. 20, p. 8038–8045, 2005.

CONTRERAS, M. et al. Protein extraction from agri-food residues for integration in biorefinery: Potential techniques and current status. **Bioresource Technology**, v. 280, p. 459–477, 2019. Disponível em: <https://doi.org/10.1016/j.biortech.2019.02.040>.

DADON, S. B.; ABBO, S.; REIFEN, R. Leveraging traditional crops for better nutrition and health - The case of chickpea. **Trends in Food Science & Technology**, v. 64, p. 39–47, 2017. Disponível em: <http://dx.doi.org/10.1016/j.tifs.2017.04.002>.

DAY, L. Proteins from land plants - Potential resources for human nutrition and food security. **Trends in Food Science & Technology**, v. 32, n. 1, p. 25–42, 2013. Disponível em: <http://dx.doi.org/10.1016/j.tifs.2013.05.005>.

DELFINO, R. A.; CANNIATTI-BRAZACA, S. G. Interação de polifenóis e proteínas e o efeito na digestibilidade proteica de feijão comum (Phaseolus vulgaris L.) cultivar Pérola. **Ciencia E Tecnologia De Alimentos**, v. 30, n. 2, p. 308–312, 2010.

DIAS, D. R. et al. In vitro protein digestibility of enzymatically pre-treated bean (Phaseolus vulgaris L.) flour using commercial protease and Bacillus sp. protease. **Food Science and Technology**, v. 30, n. 1, p. 94–99, 2010. Disponível em:

<http://www.scielo.br/scielo.php?script=sci_arttext&pid=S0101-20612010000100014&lng=en&nrm=iso&tlng=en>.

DING, Q. et al. Impact of ultrasound pretreatment on hydrolysate and digestion products of grape seed protein. **Ultrasonics Sonochemistry**, v. 42, p. 704–713, 2018. Disponível em: <https://doi.org/10.1016/j.ultsonch.2017.11.027>.

EL-ADAWY, T. A. Effect of sesame seed proteins supplementation on the nutritional, physical, chemical and sensory properties of wheat flour bread. **Plant Foods for Human Nutrition**, v. 48, n. 4, p. 311–326, 1995.

EL-ADAWY, T. A.; TAHA, K. M. Characteristics and composition of different seed oils and flours. **Food Chemistry**, v. 74, n. 1, p. 47–54, 2001.

ESPINOSA-PÁEZ, E. et al. Increasing antioxidant activity and protein digestibility in Phaseolus vulgaris and Avena sativa by fermentation with the Pleurotus ostreatus Fungus. **Molecules**, v. 22, n. 12, p. 1–11, 2017.

FAKOLADE, P. O. Effect of age on physico-chemical, cholesterol and proximate composition of chicken and quail meat. **African Journal of Food Science**, v. 9, n. 4, p. 182–186, 2015.

FANG, Z.; BHANDARI, B. Encapsulation of polyphenols e a review. **Trends in Food Science and Technology**, v. 21, n. 10, p. 510–523, 2010. Disponível em: <http://dx.doi.org/10.1016/j.tifs.2010.08.003>.

FANTOZZI, P. Grape seed: A potential source of protein. **Journal of the American Oil Chemists' Society**, v. 58, n. 12, p. 1027–1031, 1981.

FAO. Food energy - Methods of analysis and conversion factors. **FAO Food and Nutrition Paper**, n. 77, p. 7–17, 2003.

FAWALE, O. S. et al. Effects of cooking and fermentation on the chemical composition, functional, and antinutritional properties of kariya (Hildergardia barteri) seeds. **Food Science and Nutrition**, v. 5, n. 6, p. 1106–1115, 2017.

FRIAS, J. et al. Assessment of the nutritional quality of raw and extruded Pisum sativum L. var. laguna seeds. **LWT - Food Science and Technology**, v. 44, n. 5, p. 1303–1308, jun. 2011.

GAHFOOR, K. et al. Effects of roasting on bioactive compounds, fatty acid, and mineral composition of chia seed and oil. **Journal of Food Processing and Preservation**, v. 42, n. 10, p. 1–7, 2018.

GARCÍA-AGUILAR, L. et al. Nutritional value and volatile compounds of black

cherry (Prunus serotina) seeds. **Molecules**, v. 20, p. 3479–3495, 2015.

GAVELLE, E. et al. Protein adequacy is primarily a matter of protein quantity, not quality: Modeling an increase in plant: Animal protein ratio in French adults. **Nutrients**, v. 9, n. 12, p. 1333–1346, 2017.

GENÇDAĞ, E.; GÖRGÜÇ, A.; YILMAZ, F. M. Recent advances in the recovery techniques of plant-based proteins from agro-industrial by-products. **Food Reviews International**, v. 37, n. 4, p. 1–22, 2020.

GIACOMINO, S. et al. Extruded flaxseed meal enhances the nutritional quality of cereal-based products. **Plant Foods for Human Nutrition**, v. 68, n. 2, p. 131–136, 2013.

GIAMI, S. Y. Effect of fermentation on the seed proteins, nitrogenous constituents, antinutrients and nutritional quality of fluted pumpkin (Telfairia occidentalis Hook). **Food Chemistry**, v. 88, n. 3, p. 397–404, 2004.

GIAMI, S. Y.; ISICHEI, I. Preparation and properties of flours and protein concentrates from raw, fermented and germinated fluted pumpkin (Telfairia occidentalis Hook) seeds. **Plant Foods for Human Nutrition**, v. 54, n. 1, p. 67–77, 1999.

GILANI, G. S. Background on international activities on protein quality assessment of foods. **British Journal of Nutrition**, v. 108, p. S168–S182, 2012.

GILANI, G. S.; LEE, N. Sources of food-grade protein. **Encyclopedia of Food Sciences and Nutrition**, p. 4873–4879, 2003.

GILANI, G. S.; SEPEHR, E. Protein digestibility and quality in products containing antinutritional factors are adversely affected by old age in rats. **The Journal of Nutrition**, v. 133, n. 1, p. 220–225, 2003. Disponível em: <http://jn.nutrition.org/content/133/1/220%5Cnhttp://jn.nutrition.org/content/133/1/220.full.pdf%5Cnhttp://jn.nutrition.org/content/133/1/220.short%5Cnhttp://www.ncbi.nlm.nih.gov/pubmed/12514294>.

GILANI, G. S.; XIAO, C. W.; COCKELL, K. A. Impact of antinutritional factors in food proteins on the digestibility of protein and the bioavailability of amino acids and on protein quality. **British Journal of Nutrition**, v. 108, p. S315–S332, 2012.

GOLBERG, A. et al. Energy-efficient biomass processing with pulsed electric fields for bioeconomy and sustainable development. **Biotechnology for Biofuels**, v. 9, n. 94, p. 1–22, 2016.

GÖRGÜÇ, A.; BIRCAN, C.; YILMAZ, F. M. Sesame bran as an unexploited by-product: Effect of enzyme and ultrasound-assisted extraction on the recovery of protein and

antioxidant compounds. **Food Chemistry**, v. 283, p. 637–645, 2019. Disponível em: <https://doi.org/10.1016/j.foodchem.2019.01.077>.

GRASSO, A. C. et al. Older consumers' readiness to accept alternative, more sustainable protein sources in the European Union. **Nutrients**, v. 11, n. 8, 2019.

GUASCH-FERRÉ, M. et al. Associations of monounsaturated fatty acids from plant and animal sources with total and cause-specific mortality in two US prospective cohort studies. **Circulation Research**, v. 124, n. 8, p. 1266–1275, 2019a.

GUASCH-FERRÉ, M. et al. Meta-analysis of randomized controlled trials of red meat consumption in comparison with various comparison diets on cardiovascular risk factors. **Circulation**, v. 139, n. 15, p. 1828–1845, 2019b.

HABIBA, R. A. Changes in anti-nutrients, protein solubility, digestibility, and HCl-extractability of ash and phosphorus in vegetable peas as affected by cooking methods. **Food Chemistry**, v. 77, n. 2, p. 187–192, 2002.

HAGEN, S. R.; FROST, B.; AUGUSTIN, J. Precolumn phenylisothiocyanate derivatization and liquid chromatography of aminoacids in food. **Journal of the Association of Official Analytical Chemists**, v. 72, n. 6, p. 912–916, 1989.

HAN, S.; CHEE, K.; CHO, S. Nutritional quality of rice bran protein in comparison to animal and vegetable protein. **Food Chemistry**, v. 172, p. 766–769, 2015. Disponível em: <http://dx.doi.org/10.1016/j.foodchem.2014.09.127>.

HARTMANN, C.; SIEGRIST, M. **Consumer perception and behaviour regarding sustainable protein consumption: A systematic reviewTrends in Food Science and Technology**, 2017. .

HAUG, W.; LANTZSCH, H. Sensitive method for the rapid determination of phytate in cereals and cereal products. **Journal of the Science of Food and Agriculture**, v. 34, n. 12, p. 1423–1426, 1983.

HE, X. et al. Effects of high pressure on the physicochemical and functional properties of peanut protein isolates. **Food Hydrocolloids**, v. 36, p. 123–129, 2014. Disponível em: <http://dx.doi.org/10.1016/j.foodhyd.2013.08.031>.

HELLEBOIS, T. et al. Structure conformation, physicochemical and rheological properties of flaxseed gums extracted under alkaline and acidic conditions. **International Journal of Biological Macromolecules**, v. 192, n. October, p. 1217–1230, 2021. Disponível em: <https://doi.org/10.1016/j.ijbiomac.2021.10.087>.

HSU, H. W. et al. A multienzime technique for estimating protein digestibility.

Journal of Food Science, v. 42, p. 1269–1273, 1977.

HUGHES, G. J. et al. Protein digestibility-corrected amino acid scores (PDCAAS) for soy protein isolates and concentrate: Criteria for evaluation. **Journal of Agricultural and Food Chemistry**, v. 59, n. 23, p. 12707–12712, 2011.

HUGHES, G. J. et al. Initial investigation of dietitian perception of plant-based protein quality. **Food Science and Nutrition**, v. 2, n. 4, p. 371–379, 2014.

HUSSAIN, S. et al. Biochemical and nutritional evaluation of unleavened flat breads fortified with healthy flaxseed. **International Journal of Agriculture and Biology**, v. 14, n. 2, p. 190–196, 2012.

IZYDORCZYK, M.; CUI, S. W.; WANG, Q. Polysaccharide gums: Scructure, functional properties, and applications. In: CUI, S. W. (Ed.). **Food Carbohydrates: Chemistry, Physical Properties, and Applications**. [s.l.] CRC Press, 2005. p. 432.

JIA, W. et al. Assessing functional properties of rapeseed protein concentrate versus isolate for food applications. **Innovative Food Science & Emerging Technologies**, v. 68, n. October 2020, p. 102636, 2021. Disponível em: <https://doi.org/10.1016/j.ifset.2021.102636>.

JOURDAN, G. A.; NOREA, C. P. Z.; BRANDELLI, A. Inactivation of trypsin inhibitor activity from Brazilian varieties of beans (Phaseolus vulgaris L.). **Food Science and Technology International**, v. 13, n. 3, p. 195–198, 2007.

KAJLA, P.; SHARMA, A.; SOOD, D. R. Flaxseed — A potential functional food source. **Journal of Food Science and Technology**, v. 52, n. 4, p. 1857–1871, 2015.

KAKADE, M. L. et al. Determination of trypsin inhibitor activity of soy products: A collaborative analysis of an improved procedure. **American Association of Cereal Chemists**, v. 51, p. 376–382, 1974.

KALPANADEVI, V.; MOHAN, V. R. Effect of processing on antinutrients and in vitro protein digestibility of the underutilized legume, Vigna unguiculata (L.) Walp subsp. unguiculata. **LWT - Food Science and Technology**, v. 51, n. 2, p. 455–461, 2013. Disponível em: <http://dx.doi.org/10.1016/j.lwt.2012.09.030>.

KAMEL, B. S.; DAWSON, H.; KAKUDA, Y. Characteristics and composition of melon and grape seed oils and cakes. **Journal of the American Oil Chemists' Society**, v. 62, n. 5, p. 881–883, 1985.

KAMELA, A. L. S. et al. Influence of processing methods on proximate composition and dieting of two Amaranthus species from West Cameroon. **International Journal of Food Science**, v. 2016, p. 1–8, 2016.

KHALID, E. K.; BABIKER, E. E.; EL TINAY, A. H. Solubility and functional properties of sesame seed proteins as influenced by pH and/or salt concentration. **Food Chemistry**, v. 82, n. 3, p. 361–366, 2003.

KHALIL, A. H.; MANSOUR, E. H. The effect of cooking, autoclaving and germination on the nutritional quality of faba beans. **Food Chemistry**, v. 54, p. 177–182, 1995.

KNISKERN, M. A.; JOHNSTON, C. S. Protein dietary reference intakes may be inadequate for vegetarians if low amounts of animal protein are consumed. **Nutrition**, v. 27, n. 6, p. 727–730, 2011. Disponível em: <http://dx.doi.org/10.1016/j.nut.2010.08.024>.

KONIECZNY, D. et al. Nutritional properties of pea protein-enriched flour treated with different proteases to varying degrees of hydrolysis. **Cereal Chemistry**, v. 97, n. 2, p. 429–440, 2020.

KOTECKA-MAJCHRZAK, K. et al. Oilseed proteins – Properties and application as a food ingredient. **Trends in Food Science and Technology**, v. 106, n. August, p. 160–170, 2020. Disponível em: <https://doi.org/10.1016/j.tifs.2020.10.004>.

KRAUSE, J.; SCHULTZ, M.; DUDEK, S. Effect of extraction conditions on composition, surface activity and rheological properties of protein isolates from flaxseed (Linum usitativissimum L). **Journal of the Science of Food and Agriculture**, v. 82, n. 9, p. 970–976, 2002.

KUMAR, M. et al. Tomato (Solanum lycopersicum L.) seed: A review on bioactives and biomedical activities. **Biomedicine & Pharmacotherapy**, v. 142, p. 112018, 2021.

KUMARI, B. et al. Recent advances on application of ultrasound and pulsed electric field technologies in the extraction of bioactives from agro-industrial by-products. **Food and Bioprocess Technology**, v. 11, n. 2, p. 223–241, 2018.

KYRIAKOPOULOU, K.; DEKKERS, B.; VAN DER GOOT, A. J. Plant-Based Meat Analogues. In: GALANAKIS, C. M. (Ed.). **Sustainable Meat Production and Processing**. Amsterdam, Netherlands: Elsevier Inc., 2019. p. 103–126.

LAKSHMI, A. J.; KAUL, P. Nutritional potential, bioaccessibility of minerals and functionality of watermelon (Citrullus vulgaris) seeds. **LWT - Food Science and Technology**, v. 44, n. 8, p. 1821–1826, 2011. Disponível em: <http://dx.doi.org/10.1016/j.lwt.2011.04.001>.

LAMBEAU, K. V.; MCRORIE, J. W. Fiber supplements and clinically proven health benefits: How to recognize and recommend an effective fiber therapy. **Journal of the American Association of Nurse Practitioners**, v. 29, n. 4, p. 216–223, 2017.

LAN, Y. et al. Physicochemical properties and aroma profiles of flaxseed proteins

extracted from whole flaxseed and flaxseed meal. **Food Hydrocolloids**, v. 104, n. January, p. 105731, 2020. Disponível em: <https://doi.org/10.1016/j.foodhyd.2020.105731>.

LAZOS, E. S. Certain functional properties of defatted pumpkin seed flour. **Plant Foods for Human Nutrition**, v. 42, n. 3, p. 257–273, 1992.

LEE, J. Y. et al. Effect of gamma irradiation and autoclaving on sterilization and amino acids digestibility of diets for specific pathogen free mini-pigs containing either soybean meal or whey protein. **Livestock Science**, v. 149, n. 1–2, p. 201–207, 2012. Disponível em: <http://dx.doi.org/10.1016/j.livsci.2012.07.010>.

LI, J. et al. Microwave-assisted approach for the rapid enzymatic digestion of rapeseed meal. **Food Science and Biotechnology**, v. 19, n. 2, p. 463–469, 2010.

LI, M. et al. Functional properties of protein isolates from bell pepper (Capsicum annuum L. var. annuum) seeds. **LWT - Food Science and Technology**, v. 97, p. 802–810, 2018.

LIU, C. et al. Molecular and functional properties of protein fractions and isolate from cashew nut (Anacardium occidentale L.). **Molecules**, v. 23, n. 393, p. 1–15, 2018.

LIU, K.; ZHENG, J.; CHEN, F. Relationships between degree of milling and loss of vitamin B, minerals, and change in amino acid composition of brown rice. **LWT - Food Science and Technology**, v. 82, p. 429–436, 2017. Disponível em: <http://dx.doi.org/10.1016/j.lwt.2017.04.067>.

LLOPART, E. E. et al. Effects of extrusion conditions on physical and nutritional properties of extruded whole grain red sorghum (sorghum spp). **International Journal of Food Sciences and Nutrition**, v. 65, n. 1, p. 34–41, 2014.

LÓPEZ, D. N. et al. Amaranth, quinoa and chia protein isolates: Physicochemical and structural properties. **International Journal of Biological Macromolecules**, v. 109, p. 152–159, 2018. Disponível em: <http://dx.doi.org/10.1016/j.ijbiomac.2017.12.080>.

LOVATTO, N. M. et al. Phosphorylated protein concentrate pumpkin seed (Cucurbita moschata): Optimization by response surface methodology and nutritional characterization. **Ciencia Rural**, v. 50, n. 2, p. 1–10, 2020.

LQARI, H. et al. Lupinus angustifolius protein isolates: Chemical composition, functional properties and protein characterization. **Food Chemistry**, v. 76, n. 3, p. 349–356, 2002.

LUSAS, E. W.; RHEE, K. C. Soy protein processing and utilization. In: **Practical Handbook of Soybean Processing and Utilization**. [s.l.] AOCS Press, 1995. p. 117–160.

MACIEL, L. G. et al. The potential of the pecan nut cake as an ingredient for the food industry. **Food Research International**, v. 127, n. October 2019, p. 108718, 2020. Disponível em: <https://doi.org/10.1016/j.foodres.2019.108718>.

MANSOUR, E. H. et al. Effect of processing on antinutritive factors and nutritive value of rapeseed products. **Food Chemistry**, v. 47, p. 247–252, 1993a.

MANSOUR, E. H. et al. Evaluation of pumpkin seed (Cucurbita pepo, Kakai 35) as a new source of protein. **Acta Alimentaria**, v. 22, n. 1, p. 3–13, 1993b.

MANTOANI, A. C.; PESSATO, T. B.; TAVANO, O. L. Baixa digestibilidade proteica e presença de antinutricionais em produtos tipo mix de cereais. **Brazilian Society of Food Nutrition**, v. 38, n. 3, p. 245–255, 2013.

MARAMBE, H. K.; SHAND, P. J.; WANASUNDARA, J. P. D. In vitro digestibility of flaxseed (Linum usitatissimum L.) protein: Effect of seed mucilage, oil and thermal processing. **International Journal of Food Science and Technology**, v. 48, n. 3, p. 628–635, 2013.

MARPALLE, P. et al. Nutritional characterization and oxidative stability of α-linolenic acid in bread containing roasted ground flaxseed. **LWT - Food Science and Technology**, v. 61, n. 2, p. 510–515, 2015. Disponível em: <http://dx.doi.org/10.1016/j.lwt.2014.11.018>.

MARTÍNEZ-FLORES, H. E. et al. Functional characteristics of protein flaxseed concentrate obtained applying a response surface methodology. **Journal of Food Science**, v. 71, n. 8, 2006.

MATHAI, J. K.; LIU, Y.; STEIN, H. H. Values for digestible indispensable amino acid scores (DIAAS) for some dairy and plant proteins may better describe protein quality than values calculated using the concept for protein digestibility-corrected amino acid scores (PDCAAS). **British Journal of Nutrition**, v. 117, n. 4, p. 490–499, 2017.

MATTILA, P. et al. Nutritional value of commercial protein-rich plant products. **Plant Foods for Human Nutrition**, v. 73, n. 2, p. 108–115, 2018.

MECHI, R.; CANIATTI-BRAZACA, S. G.; ARTHUR, V. Avaliação química, nutricional e fatores antinutricionais do feijão preto (Phaseolus vulgaris L.) irradiado. **Ciência e Tecnologia de Alimentos**, v. 25, n. 1, p. 109–114, 2005.

MIRMOGHTADAIE, L.; ALIABADI, S. S.; HOSSEINI, S. M. **Recent approaches in physical modification of protein functionalityFood Chemistry**, 2016. .

MONTOYA, C. A. et al. In vitro and in vivo protein hydrolysis of beans (Phaseolus

vulgaris) genetically modified to express different phaseolin types. **Food Chemistry**, v. 106, n. 3, p. 1225–1233, 2008.

MU, T.; SUN, H.; WANG, C. **Sweet Potato Processing Technology**. [s.l.] Elsevier Inc., 2017.

MULTARI, S.; STEWART, D.; RUSSELL, W. R. Potential of fava bean as future protein supply to partially replace meat intake in the human diet. **Comprehensive Reviews in Food Science and Food Safety**, v. 14, n. 5, 2015.

MURCIA, M. A. et al. Proximate composition and vitamin E levels in egg yolk: Losses by cooking in a microwave oven. **Journal of the Science of Food and Agriculture**, v. 79, n. 12, p. 1550–1556, 1999.

NADATHUR, S. R.; WANASUNDARA, J. P. D.; SCANLIN, L. Proteins in the diet: Challenges in feeding the global population. In: **Sustainable Protein Sources**. Amsterdam: Elsevier Inc., 2016. p. 1–20.

NIJDAM, D.; ROOD, T.; WESTHOEK, H. The price of protein: Review of land use and carbon footprints from life cycle assessments of animal food products and their substitutes. **Food Policy**, v. 37, n. 6, p. 760–770, 2012. Disponível em: <http://dx.doi.org/10.1016/j.foodpol.2012.08.002>.

NIKMARAM, N. et al. **Effect of extrusion on the anti-nutritional factors of food products: An overviewFood Control**, 2017. .

NOSWORTHY, M. G. et al. Effect of processing on the in vitro and in vivo protein quality of yellow and green split peas (Pisum sativum). **Journal of Agricultural and Food Chemistry**, v. 65, p. 7790–7796, 2017a.

NOSWORTHY, M. G. et al. Impact of processing on the protein quality of pinto Bean (Phaseolus vulgaris) and buckwheat (Fagopyrum esculentum Moench) flours and blends, as Determined by in vitro and in vivo methodologies. **Journal of Agricultural and Food Chemistry**, v. 65, p. 3919–3925, 2017b.

NOSWORTHY, M. G. et al. Effect of processing on the in vitro and in vivo protein quality of beans (Phaseolus vulgaris and Vicia faba). **Nutrients**, v. 10, n. 671, p. 1–13, 2018a.

NOSWORTHY, M. G. et al. Effect of processing on the in vitro and in vivo protein quality of red and green lentils (Lens culinaris). **Food Chemistry**, v. 240, p. 588–593, 2018b. Disponível em: <https://doi.org/10.1016/j.foodchem.2017.07.129>.

NOSWORTHY, M. G.; HOUSE, J. D. Factors influencing the quality of dietary proteins: Implications for pulses. **Cereal Chemistry**, v. 94, n. 1, p. 49–57, 2017.

OJOKOH, A. O.; YIMIN, W. Effect of fermentation on chemical composition and nutritional quality of extruded and fermented soya products. **International Journal of Food Engineering**, v. 7, n. 4, 2011.

OLIVOS-LUGO, B. L.; VALDIVIA-LÓPEZ, M. Á.; TECANTE, A. Thermal and physicochemical properties and nutritional value of the protein fraction of mexican chia seed (Salvia hispanica L.). **Food Science and Technology International**, v. 16, n. 1, p. 89–96, 2010.

ONSAARD, E. Sesame proteins. **International Food Research Journal**, v. 19, n. 4, p. 1287–1295, 2012.

ONWULATA, C. I.; KONSTANCE, R. P. Extruded corn meal and whey protein concentrate: Effect of particle size. **Journal of Food Processing and Preservation**, v. 30, n. 4, p. 475–487, 2006.

PARK, S. J.; KIM, T.; BAIK, B. Relationship between proportion and composition of albumins, and in vitro protein digestibility of raw and cooked pea seeds (Pisum sativum L.). **Journal of the Science of Food and Agriculture**, v. 90, n. 10, p. 1719–1725, 2010.

PASTOR-CAVADA, E. et al. Protein isolates from two Mediterranean legumes: Lathyrus clymenum and Lathyrus annuus. Chemical composition, functional properties and protein characterisation. **Food Chemistry**, v. 122, n. 3, p. 533–538, 2010. Disponível em: <http://dx.doi.org/10.1016/j.foodchem.2010.03.002>.

PATHAK, N. et al. Value addition in sesame: A perspective on bioactive components for enhancing utility and profitability. **Pharmacognosy Reviews**, v. 8, n. 16, p. 147–155, 2014.

PATTO, M. C. V. et al. Achievements and challenges in improving the nutritional quality of food legumes. **Critical Reviews in Plant Sciences**, v. 34, n. 1–3, p. 105–143, 2015.

PENCHARZ, P. B.; ELANGO, R.; WOLFE, R. R. Recent developments in understanding protein needs – How much and what kind should we eat? 1. **Applied Physiology, Nutrition, and Metabolism**, v. 41, n. 5, p. 577–580, 2016. Disponível em: <http://www.nrcresearchpress.com/doi/10.1139/apnm-2015-0549>.

PESTANA, J. M. et al. Effects of pasteurization and ultra-high temperature processes on proximate composition and fatty acid profile in bovine milk. **American Journal of Food Technology**, v. 10, n. 6, p. 265–272, 2015.

PETRUSÁN, J.; RAWEL, H.; HUSCHEK, G. Protein-rich vegetal sources and trends in human nutrition: A review. **Current Topics in Peptide and Protein Research**, v. 17, p. 1–19, 2016.

PEYRANO, F. et al. Gelation of cowpea proteins induced by high hydrostatic pressure.

Food Hydrocolloids, v. 111, p. 106191, 2021. Disponível em: <https://doi.org/10.1016/j.foodhyd.2020.106191>.

POJIĆ, M.; MISAN, A.; TIWARI, B. Eco-innovative technologies for extraction of proteins for human consumption from renewable protein sources of plant origin. **Trends in Food Science and Technology**, v. 75, p. 93–104, 2018.

PREET, K.; PUNIA, D. Proximate composition, phytic acid, polyphenols and digestibility (in vitro) of four brown cowpea varieties. **International Journal of Food Sciences and Nutrition**, v. 51, p. 189–193, 2000.

RABRENOVIĆ, B. B. et al. The most important bioactive components of cold pressed oil from different pumpkin (Cucurbita pepo L.) seeds. **LWT - Food Science and Technology**, v. 55, n. 2, p. 521–527, 2014.

RAMACHANDRA, G.; MONTEIRO, P. V. Preliminary studies on the nutrient composition of Cassia sericea Sw. An unexploited legume seed. **Journal of Food Composition and Analysis**, v. 3, n. 1, p. 81–87, 1990.

RAYMOND, J.; INQUELLO, V.; AZANZA, J. L. The seed proteins of sunflower: Comparative studies of cultivars. **Phytochemistry**, v. 30, n. 9, p. 2849–2856, 1991.

REZIG, L. et al. Pumpkin (cucurbita maxima) seed proteins: Sequential extraction processing and fraction characterization. **Journal of Agricultural and Food Chemistry**, v. 61, n. 32, p. 7715–7721, 2013.

RIZZO, G.; BARONI, L. Soy, soy foods and their role in vegetarian diets. **Nutrients**, v. 10, n. 43, p. 1–51, 2018.

RODSAMRAN, P.; SOTHORNVIT, R. Physicochemical and functional properties of protein concentrate from by-product of coconut processing. **Food Chemistry**, v. 241, 2018.

ROGERSON, D. Vegan diets: practical advice for athletes and exercisers. **Journal of the International Society of Sports Nutrition**, v. 14, n. 36, p. 1–15, 2017.

RUIZ, G. A. **Exploring novel food proteins and processing technologies: A case study on quinoa protein and high pressure – high temperature processing**. 2016. Wageningen University, 2016.

SÁ, A. G. A. et al. Oilseed by-products as plant-based protein sources: Amino acid profile and digestibility. **Future Foods**, p. 100023, 2021. Disponível em: <https://doi.org/10.1016/j.fufo.2021.100023>.

SÁ, A. G. A. et al. Influence of emerging technologies on the utilization of plant proteins. **Frontiers in Nutrition**, v. 9, n. 809058, p. 1–18, 2022.

SÁ, A. G. A.; MORENO, Y. M. F.; CARCIOFI, B. A. M. Food processing for the improvement of plant proteins digestibility. **Critical Reviews in Food Science and Nutrition**, v. 0, n. 0, p. 1–20, 2019. Disponível em: <https://doi.org/10.1080/10408398.2019.1688249>.

SÁ, A. G. A.; MORENO, Y. M. F.; CARCIOFI, B. A. M. Plant proteins as high-quality nutritional source for human diet. **Trends in Food Science & Technology**, v. 97, n. October 2019, p. 170–184, 2020. Disponível em: <https://doi.org/10.1016/j.tifs.2020.01.011>.

SAHARAN, K.; KHETARPAUL, N. Biological utilization of vegetable peas: Effect of cooking and varietal differences. **Plant Foods for Human Nutrition**, v. 45, n. 4, p. 321–329, 1994.

SALGADO, P. R. et al. Production and characterization of sunflower (Helianthus annuus L.) protein-enriched products obtained at pilot plant scale. **LWT - Food Science and Technology**, v. 45, n. 1, p. 65–72, 2012. Disponível em: <http://dx.doi.org/10.1016/j.lwt.2011.07.021>.

SAMARANAYAKA, A. Lentil: Revival of poor man's meat. In: SUDARSHAN R. NADATHUR, J. P. D. W. AND L. S. (Ed.). **Sustainable Protein Sources**. Amsterdam, Netherlands: Elsevier Inc., 2017. p. 185–196.

SÁNCHEZ-VIOQUE, R. et al. Protein isolates from chickpea (Cicer arietinum L.): Chemical composition, functional properties and protein characterization. **Food Chemistry**, v. 64, n. 2, p. 237–243, 1999.

SARKER, A. K. et al. Comparison of cake compositions, pepsin digestibility and amino acids concentration of proteins isolated from black mustard and yellow mustard cakes. **AMB Express**, v. 5, n. 22, p. 1–6, 2015.

SARWAR, G. The protein digestibility–corrected amino acid score method overestimates quality of proteins containing antinutritional factors and of poorly digestible proteins supplemented with limiting amino acids in rats. **Journal of Nutrition**, v. 127, n. 5, p. 758–764, 1997. Disponível em: <http://jn.nutrition.org/content/127/5/758.abstract>.

SCHAAFSMA, G. The protein digestibility-corrected amino acid score (PDCAAS) - A concept for describing protein quality in foods and food ingredients: A critical review. **Journal of AOAC International**, v. 88, n. 3, p. 988–994, 2005.

SCHAAFSMA, G. Advantages and limitations of the protein digestibility-corrected amino acid score (PDCAAS) as a method for evaluating protein quality in human diets. **British Journal of Nutrition**, v. 108, p. S333–S336, 2012.

SHI, C. et al. Solid-state fermentation of corn-soybean meal mixed feed with Bacillus

subtilis and Enterococcus faecium for degrading antinutritional factors and enhancing nutritional value. **Journal of Animal Science and Biotechnology**, v. 8, n. 1, p. 1–9, 2017.

SHIM, Y. Y. et al. Flaxseed (Linum usitatissimum L.) bioactive compounds and peptide nomenclature: A review. **Trends in Food Science and Technology**, v. 38, n. 1, p. 5–20, 2014. Disponível em: <http://dx.doi.org/10.1016/j.tifs.2014.03.011>.

SHIMELIS, E. A.; RAKSHIT, S. Effect of microwave heating on solubility and digestibility of proteins and reduction of antinutrients of selected common bean (Phaseolus vulgaris L.) varieties grown in Ethiopia. **Italian Journal of Food Science**, v. 17, n. 4, p. 407–418, 2005.

SIDDHURAJU, P.; MAKKAR, H. P. S.; BECKER, K. The effect of ionising radiation on antinutritional factors and the nutritional value of plant materials with reference to human and animal food. **Food Chemistry**, v. 78, n. 2, p. 187–205, 2002.

SIDDHURAJU, P.; VIJAYAKUMARI, K.; JANARDHANAN, K. Chemical composition and protein quality of the little-known legume, velvet bean (Mucuna pruriens (L.) DC.). **Journal of Agricultural and Food Chemistry**, v. 44, n. 9, p. 2636–2641, 1996.

SOUSA, A. G. O. et al. Nutritional quality and protein value of exotic almonds and nut from the Brazilian Savanna compared to peanut. **Food Research International**, v. 44, n. 7, p. 2319–2325, 2011. Disponível em: <http://dx.doi.org/10.1016/j.foodres.2011.02.013>.

SPIES, J. R. Determination of tryptophan in proteins. **Analytical Chemistry**, v. 39, p. 1412–1415, 1967.

STONE, A. K. et al. Plant Protein Ingredients. **Encyclopedia of Food Chemistry**, v. 1, p. 229–234, 2019. Disponível em: <http://dx.doi.org/10.1016/B978-0-08-100596-5.21601-6>.

SUN-WATERHOUSE, D.; ZHAO, M.; WATERHOUSE, G. I. N. Protein modification during ingredient preparation and food processing: Approaches to improve food processability and nutrition. **Food and Bioprocess Technology**, v. 7, n. 7, p. 1853–1893, 2014.

SUN, M. et al. Nutritional assessment and effects of heat processing on digestibility of Chinese sweet potato protein. **Journal of Food Composition and Analysis**, v. 26, n. 1–2, p. 104–110, 2012. Disponível em: <http://dx.doi.org/10.1016/j.jfca.2012.03.008>.

SUNIL, L. et al. Development of health foods from oilseed cakes. **Journal of Food Processing & Technology**, v. 7, n. 11, 2016.

TAKEITI, C. Y. et al. Nutritive evaluation of a non-conventional leafy vegetable (Pereskia aculeata Miller). **International Journal of Food Sciences and Nutrition**, v. 60, n.

SUPPL. 1, p. 148–160, 2009.

TAN, S. H. et al. Canola proteins for human consumption: Extraction, profile, and functional properties. **Journal of Food Science**, v. 76, n. 1, p. R16–R28, 2011.

TANG, C. Functional properties and in vitro digestibility of buckwheat protein products: Influence of processing. **Journal of Food Engineering**, v. 82, n. 4, p. 568–576, 2007.

TERRIEN, C. Available Protein Substitutes. In: **Meat Analogs**. [s.l: s.n.]p. 65–124.

TINUS, T. et al. Particle size-starch-protein digestibility relationships in cowpea (Vigna unguiculata). **Journal of Food Engineering**, v. 113, n. 2, p. 254–264, 2012. Disponível em: <http://dx.doi.org/10.1016/j.jfoodeng.2012.05.041>.

TONTUL, I. et al. Functional properties of chickpea protein isolates dried by refractance window drying. **International Journal of Biological Macromolecules**, v. 109, p. 1253–1259, 2018.

TORREZAN, R.; FRAZIER, R. A.; CRISTIANINI, M. Efeito do tratamento sob alta pressão isostática sobre os teores de fitato e inibidor de tripsina de soja. **Boletim Centro de Pesquisa de Processamento de Alimentos**, v. 28, n. 2, p. 179–186, 2010.

TUŚNIO, A. et al. Effect of replacing soybean meal by raw or extruded pea seeds on growth performance and selected physiological parameters of the ileum and distal colon of pigs. **PLoS ONE**, v. 12, n. 1, p. 1–16, 2017.

VAGADIA, B. H. et al. Comparison of conventional and microwave treatment on soymilk for inactivation of trypsin inhibitors and in vitro protein digestibility. **Foods**, v. 7, n. 6, p. 1–14, 2018.

VAGADIA, B. H.; VANGA, S. K.; RAGHAVAN, V. Inactivation methods of soybean trypsin inhibitor - A review. **Trends in Food Science & Technology**, v. 64, p. 115–125, 2017. Disponível em: <http://dx.doi.org/10.1016/j.tifs.2017.02.003>.

VAN DER POEL, A. F. B. Effect of processing on antinutritional factors and protein nutritional value of dry beans (Phaseolus vulgaris L.). A review. **Animal Feed Science and Technology**, v. 29, n. 3–4, p. 179–208, 1990. Disponível em: <http://www.sciencedirect.com/science/article/pii/0377840190900276>.

VENDEMIATTI, A. et al. Nutritional quality of sorghum seeds: Storage proteins and amino acids. **Food Biotechnology**, v. 22, n. 4, p. 377–397, 2008.

VENUSTE, M. et al. Influence of enzymatic hydrolysis and enzyme type on the nutritional and antioxidant properties of pumpkin meal hydrolysates. **Food and Function**, v. 4, n. 5, p. 811–820, 2013.

VERONEZI, C. M.; JORGE, N. Bioactive compounds in lipid fractions of pumpkin (Cucurbita sp) seeds for use in food. **Journal of Food Science**, v. 77, n. 6, p. 653–657, 2012.

VOGELSANG-O'DWYER, M. et al. Comparison of faba bean protein ingredients produced using dry fractionation and isoelectric precipitation: Techno-functional, nutritional and environmental performance. **Foods**, v. 9, n. 3, p. 1–25, 2020.

VOUDOURIS, P. et al. Sustainable Protein Technology: An evaluation on the STW Protein programme and an outlook for the future. **Sustainable Protein Technology**, v. 1786, p. 1–60, 2017.

WAHRMUND-WYLE, J. L.; HARRIS, K. B.; SAVELL, J. W. Beef retail cut composition: 2. Proximate Analysis. **Journal of Food Composition and Analysis**, v. 13, n. 3, p. 243–251, 2000.

WANASUNDARA, J. P. D. et al. Proteins from canola/rapeseed: Current status. In: **Sustainable Protein Sources**. [s.l.] Elsevier Inc., 2017. p. 285–304.

WANG, J. et al. Physicochemical, functional and emulsion properties of edible protein from avocado (Persea americana Mill.) oil processing by-products. **Food Chemistry**, v. 288, p. 146–153, 2019.

WANG, X. et al. Subunit, amino acid composition and in vitro digestibility of protein isolates from Chinese kabuli and desi chickpea (Cicer arietinum L.) cultivars. **Food Research International**, v. 43, n. 2, p. 567–572, 2010. Disponível em: <http://dx.doi.org/10.1016/j.foodres.2009.07.018>.

WANG, Y. et al. Optimization of extrusion of flaxseeds for in vitro protein digestibility analysis using response surface methodology. **Journal of Food Engineering**, v. 85, n. 1, p. 59–64, 2008.

WHITE, J. A.; HART, R. J.; FRY, J. C. An evaluation of the Waters Pico-Tag system for the amino-acid analysis of food materials. **The Journal of Automatic Chemistry**, v. 8, n. 4, p. 170–177, 1986.

WHO/FAO/UNU EXPERT CONSULTATION. Protein and amino acid requirements in human nutrition. **World Health Organization Technical Report Series**, n. 935, p. 1–265, 2007.

WHO. Codex guideline for use of nutrition and health claims - CAC/GL 23-1997. **WHO/FAO**, n. 1997, p. 1–8, 2004. Disponível em: <http://www.fao.org/ag/humannutrition/32444-09f5545b8abe9a0c3baf01a4502ac36e4.pdf>.

WU, M. et al. The digestibility and thermal properties of fermented flaxseed protein.

International Journal of Food Engineering, v. 8, n. 4, 2012.

WU, M. et al. Effects of extrusion parameters on physicochemical properties of flaxseed snack and process optimization. **International Journal of Agricultural and Biological Engineering**, v. 8, n. 5, p. 121–131, 2015.

XIONG, T. et al. Effect of high intensity ultrasound on structure and foaming properties of pea protein isolate. **Food Research International**, v. 109, p. 260–267, 2018. Disponível em: <https://doi.org/10.1016/j.foodres.2018.04.044>.

YADAV, S.; KHETARPAUL, N. Indigenous legume fermentation: Effect on some antinutrients and in-vitro digestibility of starch and protein. **Food Chemistry**, v. 50, n. 4, p. 403–406, 1994.

YAÑEZ, E. et al. Nutritive value evaluated on rats of new cultivars of common beans (Phaseolus vulgaris) released in Chile. **Plant Foods for Human Nutrition**, v. 47, p. 301–307, 1995.

ZAYAS, J. F. Foaming properties of proteins. In: **Functionality of Proteins in Food**. [s.l: s.n.]p. 260–309.

ZHANG, B. et al. Effect of extrusion conditions on the physico-chemical properties and in vitro protein digestibility of canola meal. **Food Research International**, v. 100, p. 658–664, 2017. Disponível em: <http://dx.doi.org/10.1016/j.foodres.2017.07.060>.

ZHAO, J. et al. Functional properties of sesame seed protein prepared by two different methods. **Journal of the Chemical Society of Pakistan**, v. 34, n. 5, p. 1101–1106, 2012.

ZHAO, Q. et al. Rice dreg protein as an alternative to soy protein isolate: Comparison of nutritional properties. **International Journal of Food Properties**, v. 17, n. 8, p. 1791–1804, 2014.

ZHAO, X.; CHEN, J.; DU, F. Potential use of peanut by-products in food processing: a review. **Journal of Food Science and Technology**, v. 49, n. 5, p. 521–529, 2012.