Title: Peanut protein: an underutilized byproduct with great potential- a review

Running title: Peanut protein

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Abstract

Peanut (*Arachis hypogaea* L.) is the fourth important oilseed in the world. After oil extraction, defatted peanut is a protein-rich byproduct containing around 50% of protein that can enable the production of protein isolates (90% protein) and concentrates (70% protein). Peanut protein has an excellent amino acids profile, a desirable volatile profile, a low level of anti-nutritional factors and a steady supply. Despite these advantages, peanut protein is underutilized because of its poor functional properties caused by the native globular structure and extraction conditions. Nutritional limitations are its deficiency in methionine and lysine and its association with allergic reaction for genetically predisposed subjects. To promote the valorization of peanut protein in foods, it is very important to ensure a better functionality and a better nutritional value. This review intends to cover the properties of native peanut protein and to discuss innovative strategies including physical, chemical, and biological methods to improve the functionality and to mitigate allergens. These strategies have different degree of success in terms of protein quality and functionality, yield, sustainability, and convenience. More investigation is required to select the processing or the combination of processing to boost the application of peanut protein as a valid alternative protein.

Keywords: peanut protein, byproduct, processing, functionality, allergenicity, nutrition

1. Introduction

The quest for new protein sources has been higher than ever. Although animal proteins are primary sources with balanced amino acid profiles, plant-based protein demand has raised for several motives. Plant proteins are being explored as alternatives in food applications to feed a growing population expected to reach 10 billion by 2050. These proteins are affordable, available and have a low environmental impact compared with those deriving from animal sources. Heath concerns over the overconsumption of meat and animal-based products are also alarming for some health-conscious consumers choosing to reduce (the case of flexitarian) or to remove meat consumption from their diet as vegan or vegetarian (Boukid, 2020). It is also quite important to highlight that the ongoing pandemic COVID19 contributed into boosting the market of plant proteins due to consumers' awareness toward the relatedness between nutrition and health. Beside these advantages, plant proteins are versatile and can derive from different sources such as cereals, pulses and oilseeds. Among plants, pulses have played an important role in the quest for new vegetable protein sources. Pulses such as pea (*Pisum sativum* L.), chickpea (*Cicer arietinum* L.), fava bean (*Vicia faba* L.), or lupine (e.g. *Lupinus albus*, *Lupinus mutabilis* Sweet, *Lupinus luteus* L.) have high protein content (20 to 40%) compared to cereals like wheat (11-15%) (Boukid *et al.*, 2019). Gluten, derived from wheat, is a commodity food ingredient being used for its functional properties in bread and bakery products (Boukid *et al.*, 2018). Rice, corn kidney bean, pea and amaranth proteins are also of great importance in the gluten-free market (Shevkani and Singh, 2014; Morreale *et al.*, 2019). Oat protein is gaining lot of interest since it has high concentration of proteins (12–20%) and represents a good option for people having allergies to pulses (Boukid, 2021a). Oilseeds are valuable sources of lipid and basically processed for their edible oils leaving behind important amounts of protein-rich byproduct (Chardigny and Walrand, 2016). Proteins are usually recovered from defatted meal or oilcake and marketed as food or feed ingredients. The most produced plant protein derives from soybean (36-40% protein) (Silva *et al.*, 2018) and it dominates the market for years but its reputation as a genetically modified crop is gradually shifting the interest to other sources (MarketsandMarkets, 2019).

Peanut (*Arachis hypogae, L.*) is the fourth important oilseed in the world. Peanut is classified among the legumes family (*fabaceae*) with comparable protein content to that of pulses, and it is generally included among oilseeds due to its high oil content (Arya *et al.*, 2016). After oil extraction, defatted meal or oilcake contains up to 50–60% proteins and usually used as fertilizer, feed, or fuel (Zhao *et al.*, 2020). The nutritional value of peanut protein (PP) is high and resembles animal proteins. These proteins have low level of anti-nutritional factors, and excellent amino acid profile that can be easily digested. They have also desirable aroma and taste, and a white color to be used as a potential protein substitute (Ji *et al.*, 2017; Phongthai *et al.*, 2020). It is of great significance to extract proteins from defatted peanut and to improve its application value in the food and beverage industries (Arya *et al.*, 2016; Hu *et al.*, 2019).

Thus, the development of PP as a commodity is necessary to valorize an important byproduct and provide a high-value ingredient for various food product formulations (Jain *et al.*, 2015). Nevertheless, there are no available PP isolates (PPI) or concentrates (PPC) due to their poor functional properties such as solubility, emulsifying, foaming, and gel properties that limit its applications (Ji *et al.*, 2019). To promote the utilization of PP in foods, it is very important to ensure a better functionality and better nutritional value. Several strategies were applied including physical (e.g., heating, freezing, microwave, ultrasonic, and high-pressure), biological (hydrolysis or crosslinking enzymes) and chemical (phosphorylation and glycation) methods to improve the quality of these proteins (Ji *et al.*, 2017, 2019; Ma *et al.*, 2017). In light of these considerations, this review aims to provide an updated overview about the extraction methods and the native characteristics of PP. Furthermore, modifications strategies for improved PP were discussed with focus on mitigating protein allergenicity and enhancing functional properties.

2. Extraction

Before the extraction, peanut seeds go through a pretreatment phase that includes steps such as cleaning, dehulling and in some cases roasting. Roasting have the aim to destroy the antinutritional factors, and to reduce or eliminate the spoilage and pathogenic microorganisms of raw peanuts (Yu *et al.*, 2020). Defatting or oil removal can be done using different methods mainly hydraulic pressing, screw pressing, solvent extraction, and pre-pressing followed by solvent extraction (Tu and Wu, 2019). Milling is the most traditional approach for oil removal which requires labor and time consuming. Hydraulic press applies pressure on the seeds inside a cylinder until oil release (Sena-Moreno *et al.*, 2016). Screw press is the most efficient mechanic method, in which seeds are crushed in a rotating press barrel. Peanut flour can be defatted by repeated extraction with n-hexane until the fat content is lower than 1 %. The use of solvents can reduce extraction yield and induce undesirable changes in protein structure (Ji *et al.*, 2019).

PPI (about 70% protein) are produced from defatted peanut flour or also press cake by removing the remaining oil and water-soluble, and non-protein components. Protein concentration can be carried out by isoelectric precipitation, hexane or/ and aqueous alcohol precipitation, or ultrafiltration. Ultrafiltration membrane (30 kDa) was found also efficient to obtain PPI (72% of protein) with a good functionality such as emulsion stability index (Jain *et al.*, 2015). This can be attributed to the preservation of the native protein structure due the mildly conditions of ultrafiltration and the absence of heating or chemical addition.

PPI (about 90-95% protein) are produced by alkali solution and isoelectric precipitation. To remove water-insoluble impurities, peanut flour can be subjected to an aqueous washing phase. Then peanut flour is suspended in alkaline solution (pH 8.0–8.5). After centrifugation, the pellet is discharged, and the supernatant is precipitated under acid conditions (pH 4.5). After centrifugation, the recovered protein was neutralized (pH 7.0). Commonly, the produced PPI is dried using a spray dryer (Ochoa-Rivas *et al.*, 2017). Alkali-solution is a simple and practicable method ensuring a high protein yield and it is the most commonly used for plant protein extraction. Nevertheless, there are several drawbacks including high consumption of solvents and water, high production cost, and waste generation. The use of recent technologies such as ultrasound-assisted extraction improved extraction efficiency, reduced the processing time properties of proteins compared with those obtained using the regular alkali soluble and acid precipitation methods, but further optimization is required prior to upscaling (Sun *et al.*, 2020). Ultrasound-assisted extraction also improved the emulsifying activity index and emulsifying stability index, increased the hydrophobic amino acids and reduced molecular weight fractions compared with alkaline extraction. Ultrasound treatment changed the structure of protein by increasing of surface-to-volume ratio, and thus more protein participated in forming the interfacial layer and increased the emulsifying efficiency (Amiri *et al.*, 2018).

3. Native properties of peanut protein

3.1. Nutrition

PP contains all the 20 amino acids being the richest source of arginine (up to 12.5% of total proteins) (Arya *et al.*, 2016), which is related to several health benefits such prevention of cardiovascular disease, weight management and satiety (Smeets *et al.*, 2021). Like almost plant proteins, PP is deficient in methionine, lysine, threonine and tryptophan. The true protein digestibility of peanuts is comparable with that of animal protein (94 and 97%, respectively) and better than canola protein (84%) (FAO, 2017). The protein digestibility-corrected amino acid score (PDCAAS) of PP has been estimated to be about 0.70, which is higher than wheat (0.46) and maize (0.46). However, it is lower than soy protein (0.91) and pea protein (0.82) due the limiting amino acids (Ochoa-Rivas *et al.*, 2017).

Enzymes (e.g., flavourzyme and catalase) were also applied on PPI as a post-treatment enabling the generation of hydrolysates rich in bioactive peptides (Phongthai *et al.*, 2020). It was reported that these peptides have DPPH radical scavenging, metal chelating activity and angiotensin I-converting enzyme inhibitory effects, which indicates they may be beneficial for blood pressure regulation (Yu *et al.*, 2021). Antioxidants peptides were also identified in peanut hydrolysates such as Thr-Pro-Ala (286kDa), Ile/Leu-Pro-Ser (315kDa) and Ser-Pro (202kDa) (N *et al.*, 2014).

Peanut allergy is considered to be one of the most severe food allergies with a prevalence around 2% (Li *et al.*, 2020a). Currently, strict avoidance is the only treatment and rescue medication upon accidental exposure to peanuts since peanut is a common food ingredient (Zhang *et al.*, 2021a). PP is mainly made by storage proteins, albumins and globulins. Globulins (7S and 11S) comprise of the majority of the total protein (~ 75%) (Ji *et al.*, 2017), and it is subdivided into vicilins (7S globulins) and legumins (11S globulins) (Mueller *et al.*, 2014). Thirteen proteins have been identified as allergens in peanuts (Zhang *et al.*, 2021a). Ara h 1, 2, 3, and 6 are considered the major allergens and are often associated with severe symptoms, while Ara h 5, 7, 8, 9, 10, 11, and 12/13 are considered minor allergens since they do not cause life-threatening allergic reactions (Kim *et al.*, 2019). Ara h 8 has been shown to have cross-reactivity with from birch pollen (Bet v 1) (Palladino and Breiteneder, 2018). Ara h 5, also called peanut profilin, is associated with pollen allergy (profilins from grass and birch pollen, Phl p 12 and Bet v 2, respectively) (Zhang *et al.*, 2021a). Therefore, efficient mitigation strategies are of great interest to develop hypoallergenic PP.

3.2. Functionality

Solubility of proteins are among the most important functional properties. Naturally, due to the rigid globular structures, native PP has limited solubility (Rasheed *et al.*, 2020). Compared to soy protein, PP has high molecular weight and lacks ionizable groups resulting in poor solubility (Ji *et al.*, 2019). As a function of pH, the maximum solubility (around 80-85%) was at pH=2-3 due to the ampholytic nature of PP (Wu, 2009). At pH=4-5, the values of solubility are the minimum (up to 20%) similarly to faba bean, cowpea, and chickpea proteins due to the formation of hydrophobic aggregation (Shevkani *et al.*, 2015a; Vogelsang-O'Dwyer *et al.*, 2020). At pH (6-10), solubility gradually increased until reaching maximum values (80-90%) under basic conditions (pH> 10). As a function of extraction method, PPI showed higher solubility over all pH ranges compared to PPI, which can be attributed to the partial protein denaturation during extraction similarly to faba bean, pea, chickpea and soy protein isolates (Boye *et al.*, 2010; Boukid, 2021b; Boukid *et al.*, 2021). For instance, at neutral pH, PPC had a solubility of 80 %, compared to chickpea, faba bean, pea, kidney bean and soy protein concentrates (Shevkani *et al.*, 2015b; Martinez *et al.*, 2016; Vogelsang-O'Dwyer *et al.*, 2020). PPI has higher water holding capacity and oil binding capacity compared to those of PPC due to the high degree of degradation resulting unfolding of the polypeptide chain thereby a higher capacity to water entrapment (Jain *et al.*, 2015). Water holding capacity of peanut was found higher than faba bean but lower than soy protein isolates (Table 1). Similarly, the oil holding capacity of peanut was found higher than faba and to soy protein isolates (Tontul *et al.*, 2018).

Foaming capacities of PPI were found higher than chickpea; within the same range with cowpea, pigeon pea and lower than kidney bean, faba bean, pea, and amaranth proteins (Shevkani *et al.*, 2014; Martinez *et al.*, 2016; Mohanan *et al.*, 2020). Emulsifying capacity of PPI was compared to faba bean, pea and soy protein concentrates (Table 1). Furthermore, foaming and emulsifying capacities of the PPC were higher than those of PPI as PCC have higher number of polypeptide chains allowing more fluid to be incorporated (Yu, 2007; Jain *et al.*, 2015). Overall, several factors can influence the functionality of PP including protein concentration, protein structure, viscosity, and pH (Shevkani *et al.*, 2014). Considering the method of extraction, the functional properties of PP change significantly since processing impact protein structure. Further studies on the impact of processing on PP will provide insightful information to modulate protein functionality and thus tailor their properties to fit specific food applications.

**Table 1

4. Modification strategies for peanut protein with improved properties

4.1. Mitigation strategies to reduce adverse reaction to peanut protein

Boiling of PP resulted in Ara h 1 degradation and the aggregation of fragments resulting in increased surface hydrophobic index and a decreased content of α-helixes in rAra h 1. This suggests that the epitope lost its native structure due to the heat treatment, which reduced the allergenic nature of rAra h 1 (Tian *et al.*, 2018). Boiling and frying of peanut reduced the contents of Ara h 2, 6, and 7 as well as Ara h 8 and Ara h 9 up to 50-70% (Dhital *et al.*, 2014). This can be due to the dissolution of allergens in boiling water or oil. Roasting (> 130°C for 20 min) reduced the sensitivity of Ara h1, while over 140°C enhanced IgE-binding capacity of Ara h 1 and Ara h 2 (Zhang *et al.*, 2018). This can be due to higher level of trypsin inhibition activity in roasted peanut and the formation of new complex molecules having allergenic potential (Shah *et al.*, 2019). Cold plasma, high-pressure, gamma irradiation, and pulsed-electric field are also being explored. Cold plasma reduced antigenicity by 65% for Ara h 1 and 66% Ara h 2 (Venkataratnam *et al.*, 2020). The allergenicity could be reduced by limited enzymatic hydrolysis and high-pressure homogenization (Ma *et al.*, 2017). The combination of high pressure (500 and 600 MPa) and thermal treatment (at 75 °C) reduced IgE binding to Ara h 2 (Long *et al.*, 2016). Ara h 6 was completely degraded after being treated with gamma irradiation (Luo *et al.*, 2013). Pulsed-electric field had limited effect on the structural alteration of peanut (Ara h 2, Ara h 6) and thus on the allergenic potential (Zhang *et al.*, 2021b).

Regarding chemical modifications, Ara h 2 and Ara h 6 were reduced after a treatment with dithiothreitol followed by alkylation with iodoacetamide. This treatment altered the tertiary and secondary structure of protein due to the loss of the α-helix and the increase in the β-sheets (Apostolovic *et al.*, 2013). The complexation of polyphenols to peanut flour was found efficient in reducing the allergenic potential of PP by mitigating cell degranulation (Plundrich *et al.*, 2017). The phytic acid treatment of peanut extract resulted in the formation of a complex with Ara h 1 and Ara h 2, and reduced IgE binding of the obtained solution (Chung and Champagne, 2007). This can be explained by the ability of phytic acid to precipitate the allergen and reduce its exposure to digestive enzymes.

Enzymatic hydrolysis is a safe, no added- chemical and requires low energy inputs for the reduction of peanut immunogenicity. Enzymatic hydrolysis using papain, ficin and bromelain has been reported as an efficient strategy to decrease IgE-binding up to 85-95% (Meng *et al.*, 2020). Likewise, the hydrolysis of allergens from peanut extracts using alcalase and flavourzyme reduced 91.8% of IgE binding (R *et al.*, 2015). Hydrolyzing raw peanuts with alcalase and papain was efficient to reduce IgE binding to Ara h 1 (up 100%), Ara h 2 (up 99%), and Ara h 6 (up 88%) and Ara h 3 (up to 46%) (Mikiashvili and Yu, 2018). In addition, combining physical and enzymatic treatments drastically decreased Ara h1 61 and h2 amounts (Ma *et al.*, 2017). Cross-linking enzymes (microbial polyphenol oxidase and laccase) modified tertiary structure of PP, and increased production of IgG2a antibodies and reduced IL-13 secretion (Mihajlovic *et al.*, 2016). Microbial transglutaminase formed a compact structure and reduced surface hydrophobic index and increased steric hindrance of rAra h 1 (Hu *et al.*, 2019). As a result, the formed complex did not bind with antibodies, and consequently had reduced allergic reaction (Tian *et al.*, 2020).

4.2. Strategies for better functionality

To improve the functional characteristics of PP and to meet the additional requirements of the food industry, high-pressure microfluidization treatment enabled to increase PPI solubility. This treatment induced protein disaggregation by changing the polar environment and promoting surface hydrophobicity in aqueous dispersion (Gong *et al.*, 2019). Atmospheric cold plasma treatment decreased the degree of protein aggregation and increased the number of protein surface hydrophilic groups, thereby unfolding the protein secondary structure. This would enhance the polarity on the protein surface and generate more protein–water binding sites, thereby improving the water solubility , emulsion stability, and water holding capacity (Ji *et al.*, 2017, 2019). Multiple freeze-thaw cycles increased the carbonyl content and particle size of PPI resulting in improving the emulsifying properties. The best emulsifying ability was obtained after 3 cycles where the obtained emulsion had small particle size and uniform distribution (Feng *et al.*, 2020). Thermosonication followed by proteolysis unfolded proteins and reduced particle size resulting in a remarkable increase in protein solubility for the hydrolysates (Zhang *et al.*, 2019). Nanotechnology resulted in increasing the surface hydrophobicity of PPI compared to untreated PPI. The formed nanoparticle improved emulsion ability and stability (Ning *et al.*, 2020)

As for chemical modifications, pH-shifting (pH 2, pH 4, pH 10, and pH 12) enabled the modulation of PPI properties (Li *et al.*, 2020b). At pH 10, water holding capacity was improved due to the decreased particle size, increased solubility, free sulfhydryl group content and surface hydrophobicity, while at pH12 gel ability was lost due to protein aggregation. Phosphorylation using sodium trimetaphosphate improved emulsifying activity of PPI (Sánchez-Reséndiz *et al.*, 2018).

Partial hydrolysis of protein improved peanut functional properties (solubility foaming capacity, foam stability and emulsifying ability) due to the exposure of hydrophobic groups, liberation of ionizable groups and the formation of hydrolyzed proteins with better ability to bind oil and water (Pan *et al.*, 2017; Chen *et al.*, 2018). However, protein hydrolysates may lose functional properties, depending on the type of enzyme and degree of hydrolysis (Chen *et al.*, 2018). Enzymatic hydrolysis using papain combined with high-pressure homogenization reduced particle size and improved solubility but to less extent emulsifying, fat-binding and foaming abilities (Ma *et al.*, 2017). Extrusion pretreatment and papain-induced proteolysis increased the degree of hydrolysis and protein solubility leading to improved emulsifying ability of the hydrolysates (Chen *et al.*, 2018).

5. Conclusion

Plant-based proteins are having a momentum in the food and beverage industries, which gives room to emerging sources such as PP. The use of peanut as a source of proteins have several advantages mainly valorizing an important byproduct of peanut oil/butter industry, and thus reducing the environmental impact of this industry. PP can be a promising food ingredient due to their desirable color and flavor, and good composition of essential and non-essential amino acids. Nevertheless, PP has some cons specially their deficiency in methionine and threonine levels, which can be overcome by making blends of proteins from different sources. PP is also among the list of foods allergens but several approaches are being applied to mitigate epitopes trigging allergenic reactions. Functionally, the low solubility among other properties is limiting the use PP as a food ingredient. Nevertheless, chemical modification was found efficient in improving protein functionality but it includes the uses of chemical and generates high wastes; physical modification requires high energy consumption and it some cases increases allergen reaction; while biological modification can provide chemical-free ingredients but more investigation is required to control the outcome of the proteolytic process. Developing hurdle approaches such as enzymes and ultrasound can be promising taking into account safety, functionality, price and sustainability.

Acknowledgements

This work was supported by CERCA Programme (Generalitat de Catalunya).

Author contributions

Fatma Boukid: Conceptualization (lead); Methodology (lead); Writing-original draft (lead); Writing-review & editing (lead).

Conflict of interest

None.

Compliance with ethics requirements

This article does not contain any studies with human or animal subjects.

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