Physical and techno-functional properties of commercially available plant-based proteins

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In this study, the techno-functional and physical properties of various commercially available protein isolates and hydrolysates were compared to evaluate their potential application as alternative sources of proteins in liquid and solid food formulations. The foaming ability, foam stability, emulsifying ability, creaming stability and color of faba bean protein (FPI), pea protein (PPI), rice protein (RPI), hemp protein (HPI), and soy protein isolates (SPI) were measured. FPI and RPI possess a low coloring potential, because they have the lowest L*, a* and b* of color parameters. Of the investigated commercial protein isolates with the highest foaming ability, SPI (200% in 4% protein solutions) was significantly distinguished, followed by PPI (from 105 to 110% in 4% protein solutions), followed FPI (50%), RPI (35%) and HPI (15%). More than 60-70% of the formed foam was retained in a period of 60 min in 4% solutions. Emulsifying activity index (EAI) was the highest for SPI powder (33 m^2/g) followed by PPI, FRI, and RPI powders (from 22 to 28 m^2/g) and HPI (8 m^2/g). Soy (SPI) and pea (PPI) proteins were found to have the best foam stabilizing and emulsion stabilizing properties, as well as very good creaming and cream stabilizing ability. This information may help food formulators create a new generation of plant-based food and beverage products with improved nutritional properties.

Keywords: faba bean protein, pea protein, soy protein, rice protein, hemp protein, functional properties

INTRODUCTION

In the recent years, there has been an increased interest in the use of plant proteins as technological and functional ingredients in various food products [1]. The reasons for this shift are numerous and include growing consumer awareness of the environmental impact of food production, ethical and health concerns related to animal-based foods, and a general growing interest in eating more plants [1, 2]. Consumers are more willing to accept a new food if it resembles an already familiar food product. For this reason it is difficult to replace nutritional formulas containing animal proteins with satisfactory adequate plant-based alternatives [3]. Therefore, more plant proteins should be investigated for their functional and nutritional properties to facilitate the design of these replacement food products. Traditionally, soy protein has been the most popular plant protein for the production of food analogues, such as meat and milk alternatives, because it is cheap, available in large quantities and has very similar techno-functional properties associated with real meat and dairy products, such as high water holding capacity, ability to form semi-solid textures and ability to stabilize emulsions [4]. Additionally, soy proteins have been used in many other applications, such as baked goods, snacks and functional beverages, as well as plant-based cheeses and eggs [4]. Although soy is an established plant protein that provides a range of beneficial functions, it has some limitations [2]. There is a growing demand for protein and oil rich crops; this may change again in the future. For these reasons, it is important to establish the functional characteristics of other protein-rich plant sources in food matrices.

The functional properties of various proteins have been investigated as potential plant alternatives, including proteins from pea, chickpea, faba bean, and coconut [5]. Researchers have reported that proteins have potential in multiple food applications, including plant-based meat, pasta, and baked goods, due to their beneficial functional properties in these applications, but extensive comparisons of functional properties are lacking [5]. Most of the studies have focused on investigating the functional properties of proteins in laboratory settings, instead of commercially available protein ingredients [6]. For formulators, it is more important to establish the functional properties of commercial protein ingredients to be successfully applied in various innovative nutritional formulas [7]. A recent review highlighted some of the physicochemical and techno-functional properties of various plant proteins, including their solubility profile [1, 2].

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Foaming is one of the important techno-functional properties, which is commonly used in the food industry to produce items such as cream or ice cream [8]. During foaming, proteins are adsorbed at the air/water interface, reducing the surface tension between the air bubbles, thereby stabilizing the foam [9]. For these reasons, this study aimed to compare the physical and functional properties of commercial vegetable protein powders. The differences in foaming ability, foam stability emulsifying ability, creaming stability and color of the commercial ingredients were evaluated. This information can help food manufacturers to create a new generation of plant-based foods and beverages.

EXPERIMENTAL

Materials

The commercial proteins were purchased from the markets and have the following characteristics as origin, amino acid composition, nutritional composition, declared by the manufacturers, presented in Table 1.

METHODS

Physical properties

- *Color* Six g of powdered protein were weighed into a petri dish (60 mm × 15 mm) and the color coordinates were measured using Colorimeter 10 QC 200712. The instrument was calibrated using standard black and white tiles before sample standard black and white tiles before sample analysis. The L*, a*, and b* values of the samples were then determined. To determine the chroma (C) hue (h°), and browning index (BI), equations according to Granato and Masson were used [10].

- *Foaming abilities and foam stability* The foaming properties of the investigated plant-based proteins were studied by a stirring/shaking method. The series of concentrations of protein solutions were prepared (2%, 4%, and 8% w/w). All foam tests were performed in duplicate. Reproducibility of the results was typically expressed as mean ± 10%. The foaming ability (FA, %) was determined as an aliquot of 15 cm^3 sample solution whipped in a graduated 50 cm^3 cylinder by hand for 60 s. The foaming ability was determined by the volume increase (%) immediately after shaking and was calculated according to Cano-Medina *et al.* [11]. The foam stability (FS, %) is characterized by the volume of entrapped air still remaining in the foam after a certain period of time, t > 0. The foam stability was defined as the volume of the foam that remained after 60 min at room temperature (20°C) and was expressed as a percentage of the initial foam volume.

Table 1. Commercially available proteins studied, origin, batch number and manufacturer

Sample protein	Bach number	Producer	Abbreviation
Pea protein isolate, 85% of protein	7PPV023 28.02.2025	OstroVit Technology of nutrition	PPI1
Pea protein isolate PISANE™ C9 pea protein, 84% of protein	2022228876 04.06.2024	KUK-Austria GmbH	PPI2
SUPRO 670 IP Isolated soy protein, 92% of protein	RQ-228152, G010066352	KUK-Austria GmbH	SPI
VITESSENCE Prista P 360 Faba Bean Protein, 60% of protein	ABY2001, 25.01.2024	Ingredion UK Ltd.	FPI
Rice protein hydrolysate, 79% of protein	L:3GBLJ3, 08.08.2025	Gym Beam	OPI
HEMP protein, 49% of protein	L:3GBLJ2, 09.08 2025	Gym Beam	HPI

Table 2. The L*, a*, b* color values of plant protein powders measured using an instrumental colorimeter

	PPI1	PPI2	SPI	FPI	RPI	HPI
L*	87.2±0.1^c	84.3±0.3^c	89.9±0.2^b	93.2±0.2^a	91.7±0.1^b	62.7±0.2^d
a*	6.3±0.1^b	8.2±0.1^a	4.3±0.1^c	2.9±0.1^d	4.3±0.1^c	4.3±0.1^c
b*	21.9±0.2^b	23.1±0.2^b	18.8±0.5^c	19.3±0.3^c	17.6±0.1^d	29.8±0.2^a
C	22.8±0.2^b	24.5±0.2^b	19.3±0.5^c	19.5±0.3^c	18.1±0.1^d	30.1±0.2^a
h°	73.9±0.2^c	70.5±0.3^d	77.2±0.5^b	81.4±0.1^a	76.3±0.2^b	81.8±0.2^a
E	90.1±0.2^c	87.8±0.3^c	91.9±0.1^c	95.17±0.2^a	93.5±0.1^b	69.6±0.1^d
BI	5.2±0.1^b	7.0±0.1^a	3.5±0.1^c	2.3±0.1^d	3.4±0.1^c	5.1±0.1^b

$^{a,\,b,\,c,\,d}$ Means in each row followed by different letters are the Duncan groupings from highest to the lowest showing significant difference ($p<0.05$)

The test was performed as described by Ivanov *et al.* [12]. The foam stability was given by the parameter percentage volumetric foam stability.

- *Emulsifying properties* Emulsifying properties of the sample powders were studied using method as described by Naik *et al.* [13]. Refined sunflower oil (10 cm^3) and 4 % protein solution was homogenized using homogenizer (IKA model T18 ULTRA-TURRAX, Germany) for 1 min at room temperature. Emulsions (100 mm^3) were pipetted out at 0 and 10 min and diluted with 10 cm^3 water. The absorbance of the diluted emulsion was measured at 500 nm using water as blank. Emulsifying activity index (EAI) was expressed as square meter per gram of sample by Naik *et al.* [13], emulsion stability index (ESI) was determined by Naik *et al.* [13].
- *Creaming stability* The creaming index was measured according to a method described previously by Ma *et al.* [2] with slight modifications. Freshly prepared 50% oil-in-water emulsions (20 cm^3) were done by homogenizer (as previously described). The samples of these emulsions (20 cm^3) were then poured into 50 cm^3 sample vials (h = 9.5 cm; d = 2.5 cm) immediately after preparation and stored at 4°C until analysis. The creaming stability of the emulsions was determined over a 5 days period by using a calibre gage to measure the height of the clear serum layer (HS) formed at the bottom of the emulsions after the droplets moved upwards, as well as the total height of the emulsions (HT). The creaming index was then calculated as follows: CI (%) = (HS/HT) × 100.

Statistical analysis

Statistical analysis was performed using MS Excel 2010. The data were presented as mean values ± standard deviation (SD) from three replicates. Statistical analysis was done using ANOVA, with Tukey's range statistically significant at $p< 0.05$. The different letters within each column show significant differences according to Tukey’s test at $p <0.05$.

RESULTS AND DISCUSSION

Color is one of the initial cues that a consumer uses to evaluate the quality of a food product and so it is important to assess the potential impact of different plant proteins on food appearance. Ideally, plant protein powders should be free of residual pigments and other coloring compounds, which allow them to be used in a variety of different food products.

Therefore, instrumental colorimeter values (L*, a*, b*) were measured to quantify differences in the optical properties of the powders (Table 2). Here, high lightness (L*) and low a* and b* values are preferred, which means the powder has a white appearance with only low coloring components.The results are summerized in Table 2. The lightness value (L*) of the FPI was significantly higher than for the other protein isolates and this protein powder also had overall low coloring components. The redness (a*) of all the investigated protein isolates was significantly different from that of the soy protein isolate. Specifically, PPIs were slightly red (higher a*) whereas FPI was slightly less red (lower a*), and similar OPI and HPI. There was also a significant difference in the yellowness (b*) of the protein isolates. The HPI and PPI had the strongest yellow color (b*), followed by FPI, SPI, and then OPI.

These significant differences in color may have important implications when incorporating the proteins into different food products, such as yogurts, beverages, meat analogues, and others. For example, the adjustments are often needed to formulate meat analogues to recreate meat-like colors. Moreover, thesignificant differences in the color of guava juice have been reported after the addition of soy protein [10]. Thus, these results showed that especially FPI and OPI might be suited as a protein-rich ingredient with only low coloring properties.

An important functional property in the formulation of food products of vegetable proteins is to form and stabilize foam. Plant proteins can successfully adsorb at the air-water interface and create a protective film around the air bubbles. The ability of plant proteins to foam can be characterized by measuring the foaming capacity and the stability of the resulting foam over time [9].

This study aimed to investigate the foaming properties (Figure 1) and retention of the stability of the foam formed over a period of 60 minutes (Figure 2). Commercially available protein concentrates were tested at three different concentrations - 2, 4 and 8% w/w sample, as similar concentrations were commonly used in food products. SPI at all tested concentrations shows the highest foaming activity – from 190 to 200% (Figure 1). Furthermore, more than 60% of the foam generated was hold on within 1 h (Figure 2). Pea proteins formed over 100% foam at a concentration of 4% (Figure 1). This foam held on above 50% for 1 h (Figure 2).

The variation in foam structures among different

The variation in foam structures among different samples may have resulted from different conformations of relevant proteins such as large globulin-like proteins or by an altered ratio of foam-stabilizing and -destabilizing factors due to different extraction procedures.

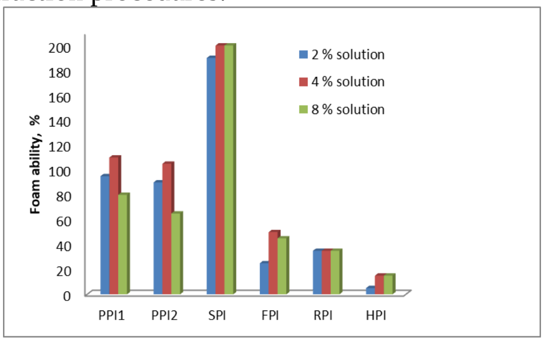

Figure 1. Foam ability (FA, %) of commercial available plant proteins

Furthermore, the variations in the foaming properties could be caused by non-proteinaceous material within the different concentrates, especially HPI and RPI containing 24% and 13% of dietary fibres.Plant protein isolates can act as emulsifiers in various food systems [5]. The type and amino acid composition of proteins determine the degree of adsorption to the surfaces of lipid droplets in the formation of emulsions. Emulsifying agents reduce the interfacial tension between the aqueous and lipid phases and form a protective coating that can prevent droplets from aggregating with each other. In many cases it is important that emulsifiers can form small uniform droplets during homogenization. For this reason, we measured the effect of protein type on its emulsifying ability in model 50% oil-water emulsions and 4% protein sample concentration. This type of information is important for commercial products because it determines how much emulsifier should be added to the product to prevent gravitational phase separation [14].

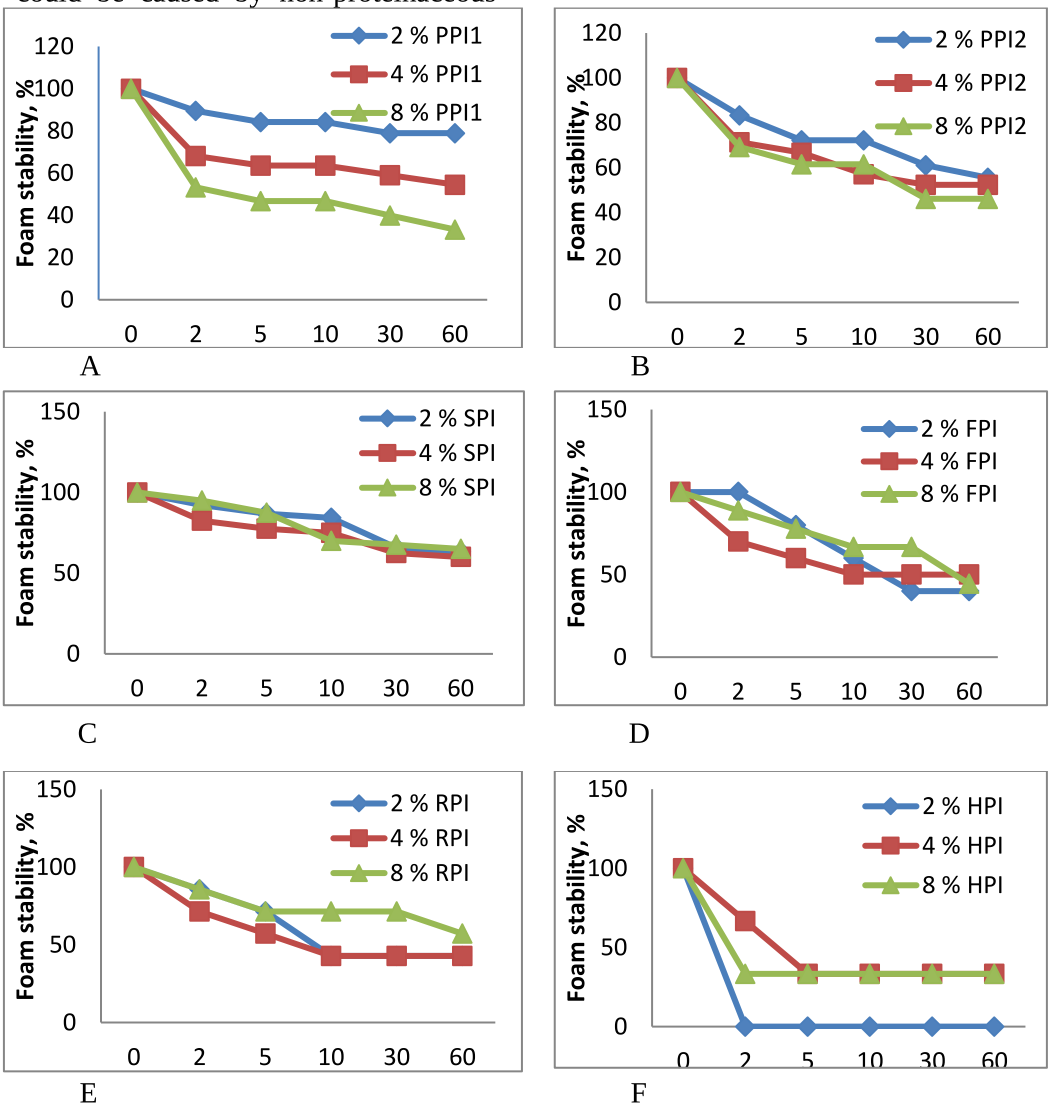

Figure 2. Foam stability (FS, %) of commercial available plant proteins; A – PPI1; B – PPI2; C – SPI; D – FPI; E – RPI; F – HPI.

Emulsifying activity index (EAI) was highest for SPI powder (33 m^2/g) followed by PPI, FRI, and RPI powder (from 22 to 28 m^2/g) and HPI (8 m^2/g). EAI indicated the area of interface (water/oil) stabilized per unit weight of powder. Similar results have been reported by different authors for pea protein, soy protein and faba protein [15-17].

Although, the EAIs were similar in value, the emulsion stability index (ESI) of the obtained emulsions was different. The highest stability of the emulsions was obtained for SPI (326 min), followed by PPI (83 min), and the protein powders HPI, RPI, and FPI (38, 35, 21 min, respectively) were of low stability.

Table 3. Emulsifying activity index (EAI) and emulsion stability index (ESI) of investigated 50% oil-water emulsions and 4% protein sample concentration

Sample	EAI, m^2/g	ESI, min
PPI1	23.58±1.25^c	83.00±0.55^b
PPI2	28.46±1.05^b	83.17±0.61^b
SPI	33.16±0.95^a	326.81±3.22^a
FPI	24.55±1.20^c	38.04±2.12^c
RPI	22.66±1.10^c	35.01±1.32^c
HPI	8.06±1.10^d	21.87±1.24^d

[a, b, c, d] Means in each column followed by different letters are the Duncan groupings from highest to the lowest showing significant difference ($p<0.05$).

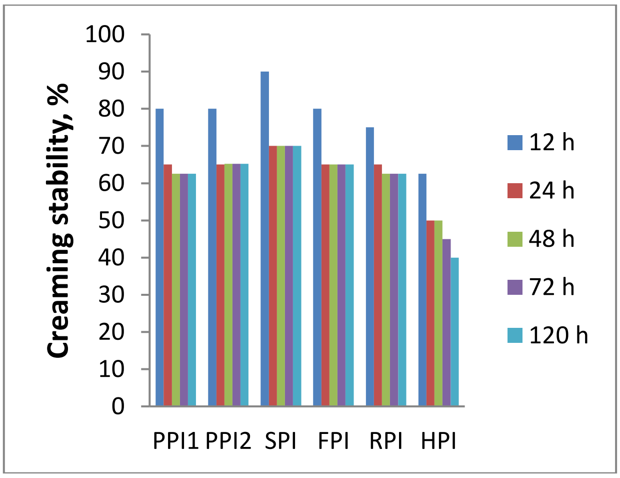

Figure 3. Creaming stability at investigated plant-based commercial available proteins for 120 h period

The rate of creaming in the prepered emulsions increases with increasing droplet size and decreasing aqueous phase viscosity. Therefore, phase separation due to this mechanism is particularly rapid in oil-in-water emulsions where the size of the oil droplet is large and the viscosity of the water phase is low [2]. Therefore, it is important in the development of the food product to ensure that plant-based proteins can maintain good stability of the formed cream to prevent phase separation. For this reason, we measured the cream stability of 50% oil-in-water emulsions containing different commercially available protein types and concentrations (4%) during storage at ambient conditions for 5 days (Fig. 3). The best creaming ability was observed with SPI (90% for fried 12 h), followed by PPI, FPI and RPI (over 65%). The formed emulsion is preserved in 65-70% during the studied period. This defines the investigated commercial proteins as good ingredients in creamy foods. The creaming of HPI emulsions is similar to the rest of the investigated emulsions in the first 12 h, but the stability of the emulsions in the investigated period significantly decreases, the observed phenomenon is not surprising since they have relatively low EIAs (Table. 2). This effect may be due to the fact, that the droplets in these emulsions are weakly flocculated and the resulting emulsions break up.

CONCLUSION

Plant-based proteins are gaining more and more popularity in various protein-enriched food products intended for dietary nutrition. The investigated pea, faba, rice, hemp and soy protein powders have significantly different functional characteristics, with good emulsifying and foaming properties possessed by soy and pea protein. All investigated proteins can form 50 % oil/water emulsions, but hemp protein stands out with the worst characteristics. The colors of the plant protein isolates were significantly different from those of the soy protein isolate, with faba bean protein powder having the lowest coloring potential. In summary, we have shown that the investigated plant proteins have relatively similar functional characteristics to soy proteins, but most of them are not as versatile as soy and therefore the specific type of protein should be selected based on the end application. This information will be very important for the design of the next generation of alternative plant-based food products. In the future, it will be important to also investigate the functionality of actual food products, as well as perform a sensory analysis of their quality characteristics.

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