AgroLife Scientific Journal - Volume 10, Number 1, 2021
ISSN 2285-5718; ISSN CD-ROM 2285-5726; ISSN ONLINE 2286-0126; ISSN-L 2285-5718

NUTRITIONAL AND FUNCTIONAL PROPERTIES OF SOME PROTEIN SOURCES

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Abstract

Nine ingredients protein sources were examined: milk protein, whey protein, Pleurotus mushroom flour, pea protein, corn protein, soy protein, oat protein, hemp protein, sea buckthorn protein in comparison with wheat flour (as control). They were subjected to physicochemical (protein, fat, ash, carbohydrates, aminoacids contents and digestibility) and functional analyzes (water absorption capacity, oil absorption capacity, foaming property and foam stability, gelling property for mixtures of 10% protein ingredient in water, emulsification capacity). The water absorption capacity ranged from 0.54-3.22 WHC. The highest oil absorption capacity was identified for milk protein sample 1.07 WHC (g oil/g sample). The foaming capacity ranged from 0% for hemp protein sample to 91.5% for milk protein sample. The emulsification capacity varied from 69.23% for the corn protein hydrolyzate sample to 54.69% for oat protein sample. Protein ingredients have different functional properties which determine their behaviour in food systems and based on which they can be used in different food applications, for improving the protein content of food products.

Key words: *water and oil absorption capacity, foaming, gelling, emulsification.*

INTRODUCTION

Plant proteins have a high nutritional quality and are industrially affordable. Previous studies have demonstrated potential functionalities of various vegetable protein sources for food applications, such as foaming (Amagliani et al., 2017; Dachmann et al., 2020), emulsifying (Ladjal-Ettoumi et al., 2015; Sarkar et al., 2016; Sharif et al., 2018; Zhang et al., 2020) and gelling properties (Lin et al., 2019; Opazo-Navarrete et al., 2018). The factors that have led to the growth of the global market for plant-derived proteins in recent years are closely linked to environmental and health issues but especially to easy access to information that has shaped food consumption trends and groups of vegetarians and flexitarians which promotes the benefits of plants (Alves & Tavares, 2019; Sá et al., 2020; Sarkar & Dickinson, 2020; Sarkar & Kaul, 2014). The use of different protein ingredients such as peas as functional ingredients in food depends on their functional attributes, such as solubility, emulsifying, foaming properties, etc. (Leqi Cui et al., 2020). Proteins perform a wide range of functions in the biological and nutritional system through the various amino acids they provide. Proteins and their derivatives can serve as a structural basis in the formulation of foods (for example thickening and gelling agents, emulsion and foam stabilizers, binders for fats, flavorings and water), as well as providing foods with special functions (for example antioxidant and antimicrobial activities) (Cao and Mezzenga, 2019; Dorica et al., 2020). Functional properties of food proteins are largely determined by their structure and their ability to interact with other food ingredients, so that they ultimately depend on their source. For example, plant proteins often exhibit inferior functionality compared to animal proteins, which leads to their limited use in food products (Jansens et al., 2019), although plant proteins with well-balanced amino acid compositions are more agriculturally-sustainable than animal proteins (Cao and Mezzenga, 2019). Most foods in which protein ingredients are added are drinks, emulsions or foams, therefore protein ingredients must have good solubility, water and oil absorption capacity, thermal stability and active surface properties (Panyam & Kilara, 1996; Amagliani et al., 2017; Alessio et al., 2020).

The aim of this study was to analyse the nutritional and functional properties of some protein sources used as ingredients in food industry in order to determine the best food applications.

MATERIALS AND METHODS

Nine ingredients protein sources were used in the experiments: milk protein (Elton Corporation SA), whey protein (Mipama Poland) *Pleurotus* mushroom flour (obtained in the laboratory from fresh mushrooms that have been washed, cut into strips, dehydrated at a temperature of 50°C until moisture reaches 8%, then ground using Retsch Mill), pea protein (Supremia Grup, Alba Iulia, Romania), corn protein (Supremia Grup, Alba Iulia, Romania), soy protein (Supremia Grup, Alba Iulia, Romania), oat protein (VTT, Finland), hemp protein (Natural Ingredients R&D SRL, Fagaras, Romania), sea buckthorn protein (Natural Ingredients R&D SRL, Fagaras, Romania) and wheat flour as control (purchased from the market).

Nutritional analysis

Physico-chemical analyzes

Physico-chemical analyzes were performed for characterization of the protein ingredients: moisture or dry matter content by means of an oven drying, ash content by calcination, determination of the protein content by Kjeldhal method, lipid content by Soxhlet extraction, total carbohydrates content (by difference).

Protein digestibility

Protein digestibility was determined *in vitro* through an enzymatic method using trypsin form porcine pancreas - Type IX-S. Trypsin solution was prepared as follows: 8 mg trypsin was dissolved in 5 ml diluted water and kept on an ice bath. The trypsin solution was freshly prepared before each test sample. The amount of sample taken corresponded to 6.25 mg protein/50 ml aqueous suspension (0.3125 x 100/g protein). The sample was placed in a water bath whose temperature was set at 37°C and the pH of the suspension was adjusted to 8.0 using 0.1 N NaOH solution or 0.1 N HCl. 5 ml of the enzyme solution was added to the sample suspension, the sample was further maintained on the water bath at 37°C with stirring. There was a rapid decrease in pH, immediately after the addition of the enzyme and the pH value was read every 1 minute for 10 minutes.

Functional analysis

Absorption capacity

The water absorption capacity is largely influenced by the granulation of each sample. In a centrifuge tube, previously weighed, 0.5 g of sample was added over 5 ml of distilled water. The suspension was stirred initially with a small vortex, then with a large vortex for 60 minutes. The suspension was centrifuged for 20 minutes at 20°C, 11000 rpm. The supernatant was removed with a pipette and the sediment tube was weighed.

a. Water absorption capacity

The water absorption capacity was expressed as g water/g sample:

$$WHC\left[\frac{g\ water}{g\ sample}\right]=\frac{W2-W1}{W0},$$

where:

W0 - the weight of the solid sample

W1 - tube + sample weight

W2 - tube + sediment weight

b. Oil absorption capacity

The oil absorption capacity was expressed as g oil/g sample:

$$WHC\left[\frac{g\ oil}{g\ sample}\right]=\frac{W2-W1}{W0},$$

where:

W0 - the weight of the solid sample

W1 - tube + sample weight

W2 - tube + sediment weight

Foaming properties and foam stability

Foam capacity (%) is the ratio of foam volume to initial volume. In a 100 ml graduated cylinder, 2 g of the sample were placed over which 50 ml of distilled water were added and mixed for 2 minutes using an ultraturex in step 4 (17500 rpm) to form the foam. The volume of the foam was measured immediately after stirring. The cylinder was left to stand for 1 hour and the volume of the foam was measured again. The stability of the foam was calculated as the ratio between the final volume of the foam and the initial volume of the foam.

Gelling property

The gelling property of protein ingredients was analyzed as follows: different types of gels were obtained from protein ingredients (10% w/v). In a 400 ml Berzelius beaker, 25 g of protein sample were weighed, over which 250 ml of water were added. The mixtures thus obtained were heated in a water bath for 45 minutes at about 80-90°C for gelling and then cooled to room temperature for 1 h. The texture properties of the gels were measured by compression tests with the Texture Analyzer (Instron Texture Analzyer, model 5944) using a 12 mm diameter cylindrical piston and a 50 N force cell. The degree of deformation was set at 50% of the initial gel height and a 2-step compression was applied at a constant speed of 4 mm/s. The force-time curves were recorded. The following texture parameters were determined: firmness (N) - maximum compressive force at which the sample has a significant resistance to deformation; adhesion - the effort required to pull the compression piston out of the sample; cohesivity - the extent to which the sample can be deformed before breaking; gumosity (N) - hardness multiplied by cohesiveness; masticability (N) - the effort required to chew the sample before swallowing.

Emulsification capacity

0.1 g of protein ingredients was weighted in a 50 ml Berzelius glass and 10 ml of distilled water were added and magnetic stirred until a homogeneous solution was obtained. The pH was adjusted to 4.5-5 with the addition of 0.1M HCL to decrease and NaOH, 1M to increase the pH. Further, 20 ml of oil were poured, 1 ml/minute, onto the solution under magnetic stirring at 2040 rpm. After the formation of the emulsion, this was transferred to 50 ml centrifuge tubes and centrifuged at 2000 rpm, 10 minutes. After centrifugation, the height of the emulsified layer (He, mm) and the total height of the liquid (Ht, mm) were measured with a ruler (Li-Tao Baia et al., 2019). The emulsifycation activity was calculated with formula:

AE (%) = He*100/Ht.

RESULTS AND DISCUSSIONS

Nutritional analysis

The physico-chemical characteristics of protein ingredients are presented in Table 1.

Table 1. Composition of the protein ingredients

Samples	Milk protein (sodium caseinate)	Whey protein	*Pleurotus* flour protein	Pea protein isolate	Corn protein hydrolyzate	Soy protein isolate	Oat protein	Hemp protein	Sea buckthorn protein	Wheat flour (control)
Moisture content (%)	9.17	6.54	8.37	6.42	2.8	6.31	9.34	4.93	7.68	11.2
Ash content (%)	6.44	6.72	5.66	5.24	6.55	3.93	3.69	5.77	4.48	0.56
Protein content (%)	76.34	73.6	16.75	74.02	24.1	82.79	44.1	29.65	14.4	10.89
Lipid content (%)	0.06	0.04	1.36	0	1.5	0.54	2.51	11.88	10.19	0.89
Total carbohydrates (%)	7.99	13.1	67.86	14.32	8.05	6.43	38.82	13.29	18.03	76.35

Soy protein isolate sample had the highest protein content: 82.79% and the sea buckthorn protein sample had the lowest protein content of 14.4%. Hemp protein and sea buckthorn sample had a higher lipid content around 11%.

Table 2. Percentage of protein digestibility for the analyzed samples

Samples	Milk protein (sodium caseinate)	Whey protein	*Pleurotus* flour protein	Pea protein isolate	Protein hydrolyzate from corn	Soy protein isolate	Oat protein	Hemp protein	Sea buckthorn protein	Wheat flour
Protein content (%)	76.34	73.6	16.75	74.02	24.1	82.79	44.1	29.65	14.4	10.89
Protein digestibility (%)	85.03	85.57	68.19	87.2	64.76	85.94	77.43	78.52	76.16	83.76

The highest digestibility (Table 2) was recorded in pea protein isolate (87.2%), soy protein isolate sample (85.94%), followed by milk protein sample (85.57%). The corn protein hydrolyzate had the lowest value of protein digestibility around 64.74%. Protein digestibility is important to predict the proportion of ingested nitrogen or amino acid made available to the organism after digestion and absorption.

Table 3. Amino acid content (from literature)

Samples	Milk protein (sodium caseinate) (g/100 g)	Whey protein (g/100g) (g/100 g protein)	*Pleurotus* flour protein (g/100 g)	Pea protein isolate (g/100g) (g/100 g protein)	Protein hydrolyzate from corn (g/100 g)	Soy protein isolate (g/100g) (g/100 g protein)	Oat protein (g/100 g)	Hemp protein (g/100g) (g/100 g protein)	Sea buckthorn protein (g/100 g)	Wheat flour (g/100 g)
Alanine	2,6	4,2 / 5,5	2,9	3,2 / 5,4	4,8	2,8 / 4,5	n.d.	1,9	0,21 / 0,03	n.d.
Arginine	2,6	1,7 / 2,7	5,2	5,9 / 8,4	1,7	4,8 / 7,8	n.d.	5,3	0,11 / 0,01	n.d.
Aspartic acid	n.d.	n.d. / 12,2	5,3	n.d. / 11,9	n.d.	n.d. / 11,8	n.d.	n.d.	4,27 / 0,04	n.d.
Glutamic acid	16,7	15,5 / 21,5	7,6	12,9 / 16,4	13,1	12,4 / 20,5	n.d.	7,4	0,19 / 0,03	n.d.
Tank	0,2	0,8 / 2,7	1,3	0,2 / 1	0,3	0,2 / 1,2	n.d.	0,2	0,03 / 0,01	n.d.
Phenylalanine	3,5	2,5 / 3,8	3	3,7 / 5,7	3,4	3,2 / 5,5	2,7	1,8	0,2 / 0,03	3,7
glycine	1,5	1,5 / 2,3	0,7	2,8 / 4	1,6	2,7 / 4,4	n.d.	2,1	0,17 / 0,01	n.d.
Histidine	1,9	1,4 / 1,6	1,8	1,6 / 2,4	1,1	1,5 / 2,5	0,9	1,1	0,14 / 0,01	1,4
Isoleucine	2,9	3,8 / 7,4	1,7	2,3 / 4,4	1,7	1,9 / 4,9	1,3	1	0,17 / 0,01	2
Leucine	7	8,6 / 12,1	6,5	5,7 / 7,6	8,8	5 / 5,6	3,8	2,6	0,02 / 0,02	5
Lysine	5,9	7,1 / 10,9	3,6	4,7 / 6,7	1	3,4 / 5,6	1,3	1,4	0,27 / 0,04	1,1
Methionine	2,1	1,8 / 2,5	0,8	0,3 / 0,9	1,1	0,3 / 1,4	0,1	1	0,02 / 0,01	0,7
Proline	7,3	4,8 / 6,1	0,8	3,1 / 4,4	5,2	3,3 / 4,9	n.d.	1,8	0,45 / 0,12	n.d.
Serine	4	4 / 6,7	2,3	3,6 / 5,4	2,9	3,4 / 5,2	n.d.	2,3	0,28 / 0,05	n.d.
Tyrosine	3,8	2,4 / 3,7	1,2	2,6 / 4	2,7	2,2 / 3,9	n.d.	1,3	0,13 / 0,02	n.d.
Threonine	3,5	5,4 / 8,8	3	2,5 / 3,8	1,8	2,3 / 3,9	1,5	1,3	0,37 / 0,06	1,8
Tryptophan	n.d.	n.d. / 1,7	n.d.	n.d. / 0,9	n.d.	n.d. / 1,3	n.d.	n.d.	0,01 / 0,01	n.d.
Valine	3,6	3,5 / 6,9	3,4	2,7 / 4,9	2,1	2,2 / 5,1	2	1,3	0,22 / 0,03	2,3

n.d. – not determined; Sea buckthorn protein (g/100 g) (*H. rhamnoides* L., *H. sinensis*). Gorissen et al., 2018; Ciesarová et al., 2020.

Milk proteins are considered a high-quality protein source taking into account their essential amino acid score, protein- digestibility corrected amino acid score and digestible indispensable amino acid scores (Mulet-Cabero et al., 2020).

Functional analysis

Absorption capacity - water and oil

The results for water and oil absorption capacity are shown in Figure 1 and Table 4. The water absorption capacity ranged from 0.54 for oat protein to 3.22 WHC (g water/g sample) for soy protein. The highest oil absorption capacity was in milk protein sample 1.07 WHC (g oil/g sample), and the lowest was recorded in the corn protein sample, respectively 0.33 WHC (g oil/g sample).

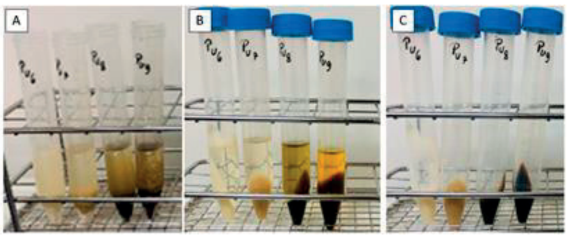

Figure 1. Oil absorption capacity: (A) the sample before centrifugation, (B) sample after centrifugation and (C) sample after removal of the supernatant (photos from experiments)

Table 4. The ability of protein ingredients to absorb water and oil

Samples	Milk protein (sodium caseinate)	Whey protein	*Pleurotus* flour protein	Pea protein isolate	Protein hydrolyzate from corn	Soy protein isolate	Oat protein	Hemp protein	Sea buckthorn protein	Wheat flour
Water absorption capacity g water/g sample	2.66	2.48	2.04	2.29	-1.00	3.23	0.54	1.27	1.19	0.78
Oil absorption capacity g oil/g sample	1.075	0.86	0.55	0.88	0.34	0.93	0.87	0.79	0.74	0.67

Water absorption capacity plays an important role in developing food texture, especially in meat products and baked dough. Protein ingredients with very high water absorption capacity may dehydrate other ingredients in a food system. Proteins with low water absorption capacity can be more sensitive to storage humidity. Oil absorption capacity (OAC) of proteins is essential in order to improve mouthfeel and flavor retention of certain food products (Khan et al., 2011). Protein ingredients with high OAC can be used in the formulation of food matrices like cake batters, mayonnaise, salad dressings and sausages (Chandi & Sogi, 2007).

Foaming properties and foam stability

Table 5. The capacity of the protein ingredients to form the foam and the foam stability

Samples	Milk protein (sodium caseinate)	Whey protein	*Pleurotus* flour protein	Pea protein isolate	Protein hydrolyzate from corn	Soy protein isolate	Oat protein	Hemp protein	Sea buckthorn protein	Wheat flour
Foaming capacity (%)	91.5	84	40	79.5	1.5	76	48.5	0	54	38
Foam stability (%)	41.5	23.5	7.5	29.5	0.5	37.5	40.5	0	22.5	7

(mean values, n = 2)

The foaming capacity ranged from 0% for hemp protein sample to 91.5% for milk protein sample. The whey protein and milk protein samples had the strongest foam stability. The wheat flour and *Pleurotus* flour protein samples had approximately the same foam capacity respectively 38-40% but also the same foam stability 7-7.5% (Table 5, Figure 2).

The property of protein ingredients to gel

Milk protein and whey protein samples had approximately the same values. The oat protein sample had slightly lower values than the previous two samples. The other samples analyzed did not form a gel (Figure 3).

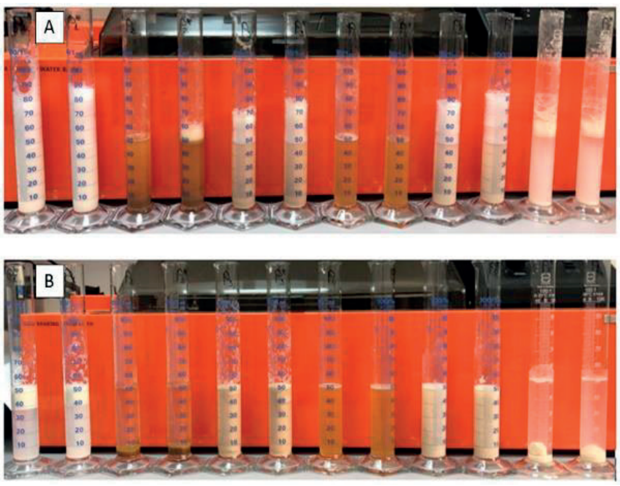

Figure 2. Foaming properties and foam stability: (A) foam formation and (B) foam stability after one hour of rest (photos from experiments)

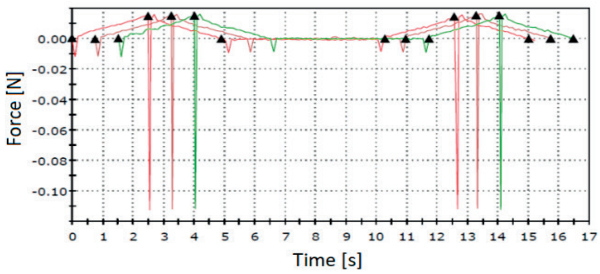

Figure 3. Gelling capacity of the sample-mixture of 10% milk protein in wheat flour

Protein emulsifying capacity

The emulsification capacity varied from 69.23% for the corn protein hydrolyzate to 54.69% for oat protein sample (Table 6).

Emulsifying ability is another important physicochemical property that may directly affect the application of proteins in food formulations (Figure 4). The emulsifying properties of proteins, in particular, are complex, usually affected by molar mass, hydrophobicity/ solubility, conformational stability and load, and physicochemical factors such as pH, ionic strength, temperature, protein concentration and profile of amino acids (Ladjal-Ettoumi et al., 2015). The samples: milk protein (sodium caseinate), whey protein, *Pleurotus* flour protein, pea protein isolate, protein hydrolyzate from corn, soy protein isolate, oat protein, hemp protein, sea buckthorn protein and wheat flour had an average emulsification capacity.

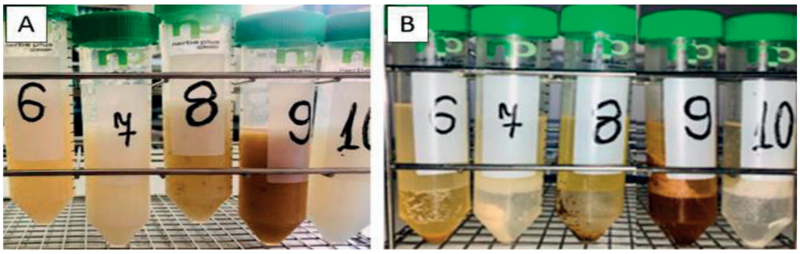

Figure 4. Protein emulsifying capacity: (A) samples before centrifugation and (B) samples after centrifugation (photos from experiments)

Table 6. Protein emulsification activity

Samples	Milk protein (sodium caseinate)	Whey protein	*Pleurotus* flour protein	Pea protein isolate	Protein hydrolyzate from corn	Soy protein isolate	Oat protein	Hemp protein	Sea buckthorn protein	Wheat flour
He (mm)	35	35	40	40	45	37	35	37	35	35
Ht (mm)	58	60	70	60	65	65	64	63	61	63
AE (%)	60.34	58.33	57.14	66.67	69.23	56.92	54.69	58.73	57.38	55.56

CONCLUSIONS

Protein ingredients have different functional properties which determine their behaviour in food systems and based on which they can be used in different food applications, for improving the protein content of food products. The selection of proteins with appropriate functional properties is vital in food formulation. However, different intrinsic, extrinsic, and environmental factors such as pH, ionic strength, and temperature affect the functional properties of proteins and these must be considered in experiments as well.

ACKNOWLEDGEMENTS

This work was supported by the project PN 19020101 through Core programme supported by the Ministry of Research, Education and Digitization and a grant of the Romanian National Authority for Scientific Research and Innovation, CCCDI-UEFISCDI, project number ERANET-CORE ORGANIC & SUSFOOD-PROVIDE 1, within PNIII.

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