

SHORT COMMUNICATION

DIGESTIBILITY OF PROTEINS FROM DIFFERENT SOURCES

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Abstract

In vitro protein digestibility can be useful for estimating protein nutritional quality. Eight protein sources were chosen to study *in vitro* protein digestibility, namely: sodium caseinate, whey, mushrooms, pea, soy, oat, hemp and sea buckthorn. Trypsin was used to achieve the enzymatic hydrolysis and the pH-drop technique was employed to determine *in vitro* protein digestibility. Substrate hydrolysis was rapidly initiated after enzyme addition in the case of pea protein, sodium caseinate, soy and whey proteins, whereas a slower pH decrease was registered in the case of mushroom proteins. The *in vitro* protein digestibility decreased in the following order: pea (87.2%) > soy (85.9%) > whey (85.6%) > caseinate (85%) > hemp (78.5%) > oat (77.5%) > sea buckthorn (76.2%) > mushrooms (68.2%). The sea buckthorn and mushrooms samples with the lowest protein contents (15.6% and 18.3%, respectively) had the lowest protein digestibility. However, no correlation between protein content and protein digestibility was found.

Keywords: protein digestibility, *in vitro*, whey, caseinate, soy, pea, mushrooms, oat, hemp, sea buckthorn

Introduction

The main role of proteins in nutrition is to provide suitable amounts of essential amino acids to meet the metabolic requirements. The quality of a protein is determined by its composition in essential amino acids (Tuan *et al.*, 1999). Besides the content of amino acids, the nutritive value of various dietary protein sources depends on their digestibility. Protein digestibility is influenced by the type of protein and by the method of food processing before ingestion (Ganapathy *et al.*, 2008). In a report from the World Health Organization, it is stated that in addition to the amino acid contents, the digestibility of proteins also needs to be taken into consideration (WHO, 2007). The type of protein sources has an impact on the degree of protein hydrolysis and on the essential amino acids' bioaccessibility,

which together account for digestibility (Le Roux *et al.*, 2020). The quality of protein together with the protein content should be taken in consideration when developing new food products (Hager *et al.*, 2013).

The evaluation of the nutritional quality of proteins can be evaluated through different approaches such as: nitrogen balance studies in humans, tests on animals, biochemical indices in animals and humans, *in vivo* and *in vitro* assays, enzymatic digestion procedures, as well as methods based on amino acid composition data (Bodwell, 1985). *In vitro* enzymatic methods are a promising tool, as they come close to reproducing the *in vivo* conditions and can be applied in a proper way to pure or crude protein sources. *In vitro* enzymatic assays of protein digestibility mimic many of the digestive processes that take place in the human stomach. The more appropriate approach should involve a two-step enzymatic system (Savoie *et al.*, 1989). The digestion of proteins occurs in two phases, defined by the site of digestion along the gastrointestinal tract: a gastric phase and an intestinal phase (Ganapathy *et al.*, 2008).

To evaluate the digestibility of proteins through enzymatic hydrolysis, one and two-step processes can be applied. The one-step approach represents the initial phase of protein digestion (peptic digestion) and includes the use of pepsin, trypsin, papain, while the two-step method combines the peptic digestion with hydrolytic pancreatic digestion using a mixture between trypsin, chymotrypsin and peptidases (Hsu *et al.*, 1977). Both peptic and pancreatic digestion steps were established according to the optimum requirements for enzyme activity in the digestive system. In general, peptic digestion is carried out at 37 °C in a hydrochloric acid solution of about pH 2 to simulate the conditions from the human stomach. For the pancreatic digestion, a phosphate buffer adjusted to pH 7.5 with an incubating temperature of 37 °C is used. In many cases, protein digestion is determined by evaluating the reduction in pH over a 10-minute time period using different enzymes (Hsu *et al.*, 1977).

The aim of this work was to investigate the protein digestibility of different protein sources using trypsin for digestion.

Materials and methods

Protein sources

The following protein sources were used: sodium caseinate (milk protein) and whey protein, which were purchased from a local provider. The protein from *Pleurotus* mushrooms was obtained by the dehydration of fresh mushrooms followed by drying (IBA Bucharest). Pea and soy protein isolates were provided by Supremia Grup (Romania). Hemp and sea buckthorn proteins were obtained from Natural Ingredients R&D SRL (Romania), while oat protein concentrate was acquired from VTT (Finland).

Compositional analysis

The compositional analysis of the protein sources was performed according to AOAC (2019) as follows: the moisture content by the gravimetric method (AOAC

925.10), the protein content by the Kjeldahl method with a conversion factor of nitrogen to protein of 6.25 (AOAC 979.09), the fat content by extraction with petroleum ether under reflux conditions in a Soxhlet (AOAC 963.15), the ash by the gravimetric method by burning at 550 °C in a furnace (AOAC 923.03), and the total carbohydrates were calculated by differences = 100 – (%moisture +%protein +%fat +%ash) (FAO, 2003).

***In vitro* protein digestibility**

The *in vitro* protein digestibility of protein sources was performed using trypsin following the method used by Hsu *et al.* (1977). The protein sample was suspended in distilled water (6.25 mg of protein/mL) and adjusted to pH 8.0 using 0.1 N NaCl and/or 0.1 N HCl, and then placed on a water bath with magnetic heating stirring at 37 °C. The trypsin enzyme solution (1.6 mg/mL; type IX-S, Sigma-Aldrich, Saint Louis, USA) was freshly prepared and kept in an ice bath. Five mL of the trypsin solution was added to the protein sample suspension at 37 °C. The decreases in pH were measured every minute for a period of 10 min using a pH meter (inoLab, WTW, Weilheim, Germany). The *in vitro* protein digestibility (Y, expressed as %) was calculated according to the equation 1, proposed by Hsu *et al.* (1977):

$$Y = 210.46 - 18.10 \times X \quad (1)$$

where X represents the pH value after 10 minutes' digestion with the trypsin solution.

Statistical analysis

The results were expressed as mean ± standard deviation and analyzed by one-way analysis of variance (ANOVA). The significance of mean differences was determined by Tukey's test (Minitab®18, UK). *P* values lower than 0.05 were considered to be significant.

Results and discussion

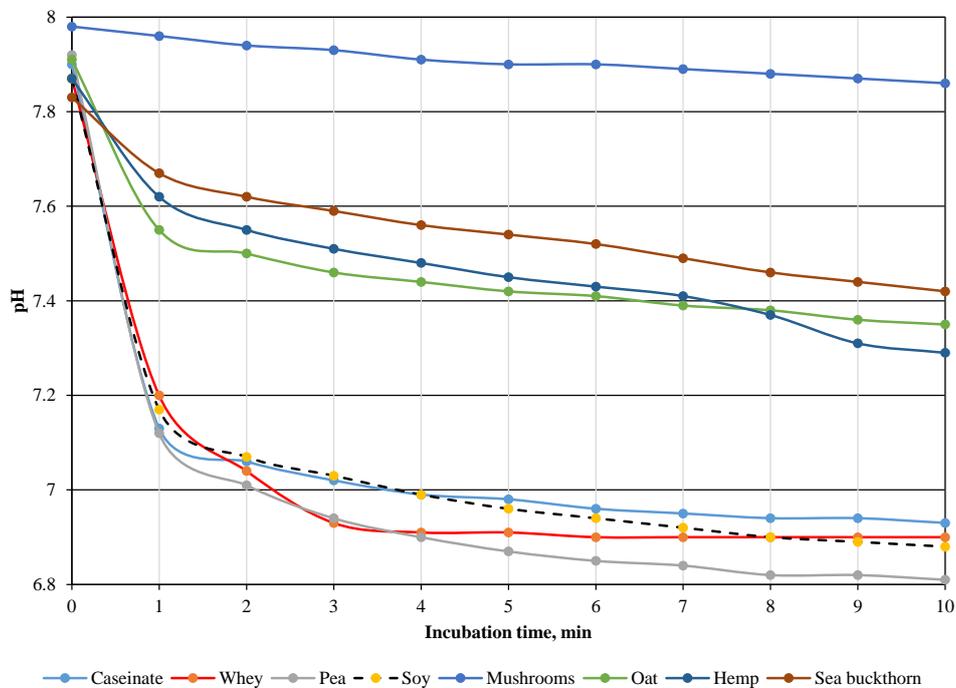
The results from the compositional analysis of the protein sources is presented in Table 1. Soy protein isolate showed significantly higher protein content ($p > 0.05$). The lowest protein content was for sea buckthorn and mushrooms samples.

Protein digestion was observed by pH change during trypsin incubation of the protein samples for 10 minutes, as it can be seen in Figure 1. After protein digestion, the peptides and amino acids released lead to pH decrease. The trypsin enzyme disrupts the protein into amino and carboxyl groups, and the liberated protons reduce the pH of the solution. Faster decreases of the pH represent higher rates of digestion and are used as an indicator of protein digestibility. About 1 minute after adding trypsin, the pH decreased rapidly for pea protein and sodium caseinate (to about 7.12), soy and whey proteins (to around 7.2). The slowest pH decrease was observed for the mushrooms sample.

Table 1. Chemical composition (% dry matter) of different protein sources.

Protein source	Protein N x 6.25	Fat	Ash	Total carbohydrates
Sodium caseinate	84.04 ± 0.10 ^b	0.06 ± 0.01 ^f	7.09 ± 0.01 ^b	8.80 ± 0.10 ^g
Whey protein	78.75 ± 0.11 ^d	0.04 ± 0.00 ^f	7.19 ± 0.02 ^a	14.01 ± 0.11 ^f
Protein from <i>Pleurotus</i> mushrooms	18.28 ± 0.07 ^g	1.48 ± 0.01 ^d	6.18 ± 0.06 ^c	74.06 ± 0.13 ^a
Pea protein isolate	79.08 ± 0.11 ^c	0.07 ± 0.01 ^f	5.59 ± 0.02 ^e	15.27 ± 0.12 ^e
Soy protein isolate	88.36 ± 0.06 ^a	0.58 ± 0.01 ^e	4.21 ± 0.01 ^g	6.86 ± 0.06 ^b
Oat protein concentrate	48.60 ± 0.13 ^e	2.77 ± 0.01 ^c	4.07 ± 0.03 ^h	44.55 ± 0.17 ^d
Hemp protein	31.25 ± 0.07 ^f	12.48 ± 0.11 ^a	6.07 ± 0.03 ^d	50.20 ± 0.18 ^c
Sea buckthorn protein	15.60 ± 0.11 ^h	11.05 ± 0.02 ^b	4.87 ± 0.01 ^f	68.49 ± 0.12 ^b

The results are expressed as mean ± standard deviation. Values followed by different letters in the same column are significantly different ($p < 0.05$).

**Figure 1.** The pH change over time obtained by incubation of different protein sources with trypsin.

The *in vitro* protein digestibility (%) of the protein sources is showed in Figure 2. A highly digestible protein was found in pea, soy, whey and caseinate samples which ranged from 87.20% ± 0.36, 85.88% ± 0.28, 85.57% ± 0.36 and 84.97% ± 0.28. Le Roux *et al.* (2020) noted that the presence of pea protein concentrate in a model infant formula showed similar or even higher *in vitro* digestibility than the milk-reference formula based on whey protein. Rachman *et al.* (2020) showed that soy protein isolate increased the protein digestibility of gluten-free pasta as compared to egg white protein.

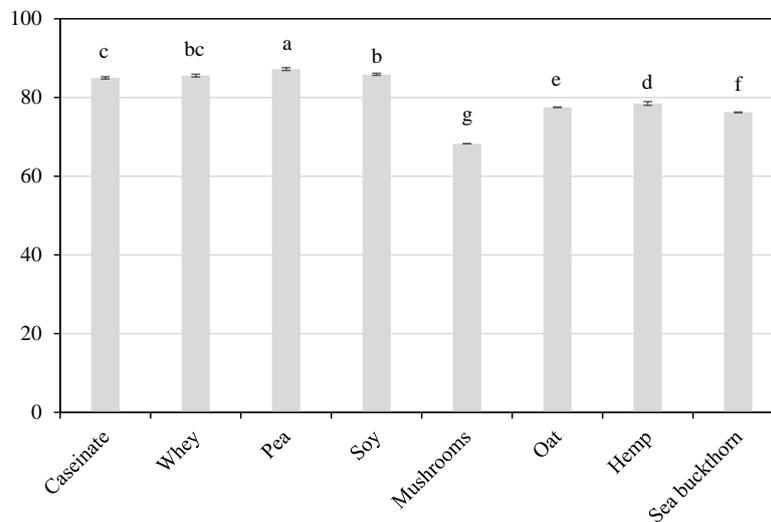


Figure 2. The *in vitro* protein digestibility (%) of protein samples.

The digestibility of the caseinate sample appeared to be lower as compared to the whey proteins. The same observation was reported by Almeida *et al.* (2015). Studies in humans showed the high true ileal digestibility of protein in milk, soy and pea to be 95%, 91% and 89%, respectively (Moughan, 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).

The sea buckthorn and mushrooms samples with the lowest protein content (15.6% and 18.3%, respectively) had the lowest protein digestibility ($p < 0.05$). The soy and whey samples did not significantly differ ($p > 0.05$) in protein digestibility. However, no correlation between the protein content and the protein digestibility could be found. Rachman *et al.* (2020) showed that protein enriched pasta with similar or higher protein content had a lower protein digestibility as compared to control. The lower protein digestibility was explained by the presence of other compounds such as fiber or phenolics which can inhibit protein digestion during the enzymatic treatment.

Hemp protein is a good source of digestible amino acids, presenting comparable amino acid profiles to egg white and soy bean, which are considered as high-quality protein (Tang *et al.*, 2006). Using a static model of gastrointestinal digestion, which included a final step with purified porcine intestinal brush border membrane vesicles, Mamone *et al.* (2019) showed a high degree of digestibility for hemp flour and hemp protein isolate. In this study, hemp protein showed higher protein digestibility when compared to the oat, sea buckthorn and mushrooms samples, but lower than the pea, soy, whey and caseinate samples.

A protein which is highly digestible could indicate a good quality protein. Food processing can affect protein digestibility. For example, the *in vitro* protein digestibility of cowpea significantly increased after pressure cooking and boiling, mainly due to the loss of antinutrients (Naidoo *et al.*, 2017). Laguna *et al.* (2017) also showed that *in vitro* pea protein digestibility was highly influenced by processing (high pressure processing) and pH (i.e. the pea protein at pH 6.2 was more digestible than at pH 3.6).

Table 2 shows research studies evaluating the protein digestibility of protein sources such as hemp, soy, pea and whey.

Table 2. Research studies evaluating the protein digestibility of different protein sources

Protein source	Protein content	Protein digestibility, %	Enzyme	References
Hemp seed protein meal	37%	84.85±0.51		
Membrane ultrafiltration protein concentrate	74%	89.0±0.26		
Isoelectric pH-precipitated protein isolate	84%	85.12±0.13	trypsin, chymotrypsin	Malomo & Aluko (2015)
Commercial hemp seed protein concentrate	70%	84.58±0.13		
Soy protein isolate	93% d.m.	86.4±0.8	pepsin	Ou <i>et al.</i> (2004)
Pea seeds (different varieties)	15.7 – 27.3%	79.9 – 83.5	trypsin, chymotrypsin, peptidase	Park <i>et al.</i> (2010)
Green pea flour	26.6%	76.26 – 85.87 (according to the drying temperature: 50 – 70 °C)	trypsin, chymotrypsin, peptidase	Gonzalez <i>et al.</i> (2020)
Whey protein supplements (USA)	61.2 – 79.5%	91.7		
Whey protein supplements (Brazil)	48.1 – 75.2%	88.4		
Soy protein powders supplement	-	83.7	pepsin, pancreatin	Almeida <i>et al.</i> (2015)
Caseinate isolate supplement	-	55.2		

Conclusions

Protein digestibility is of great interest for food industry, being one of the many indicators of protein quality. The pea, soy, whey and caseinate samples were the most digestible proteins. The protein from mushrooms had the lowest digestibility (68.2%). The knowledge of the protein digestibility in crude protein sources is a good starting point for designing new food matrices with enhanced nutritional properties.

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