Health-promoting protein-derived peptides in functional foodstuff

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The present PhD thesis's main objective is to develop tailored protein hydrolysates from food industry by-products to be used as innovative functional food ingredients. Milk whey and pea proteins were used as animal and vegetal protein sources respectively. Two of the activities included in the PhD thesis project are described. Firstly, the protein hydrolysates were characterized in terms of the degree of hydrolysis and molecular weight distribution. Secondly, the protein hydrolysates' technological and in vitro biological properties were assessed.

Impiego di peptidi bioattivi ottenuti dall’idrolisi delle proteine in alimenti funzionali

Il principale obiettivo di questa tesi di dottorato è lo sviluppo di idrolizzati proteici con specifici attributi, ottenuti da sottoprodotti delle industrie alimentari, che possano essere utilizzati come ingredienti funzionali. Le proteine del siero di latte e le proteine di pisello sono state usate come proteine di origine animale e vegetale, rispettivamente. Vengono descritte due delle attività del progetto di tesi di dottorato. Inizialmente gli idrolizzati proteici sono stati caratterizzati in termini di grado di idrolisi e distribuzione dei pesi molecolari. Inoltre, sono state valutate le proprietà tecnologiche e biologiche degli stessi.

**Key words:** whey proteins, protein hydrolysates, technological properties, biological properties

**1. Introduction**

In accordance with the PhD thesis project previously described (Di Filippo, 2022), this poster reports the main results of the two activities concerning:

(A1) the **physiochemical characterization of whey protein hydrolysates** in terms of the degree of hydrolysis (DH), and molecular weight distribution;

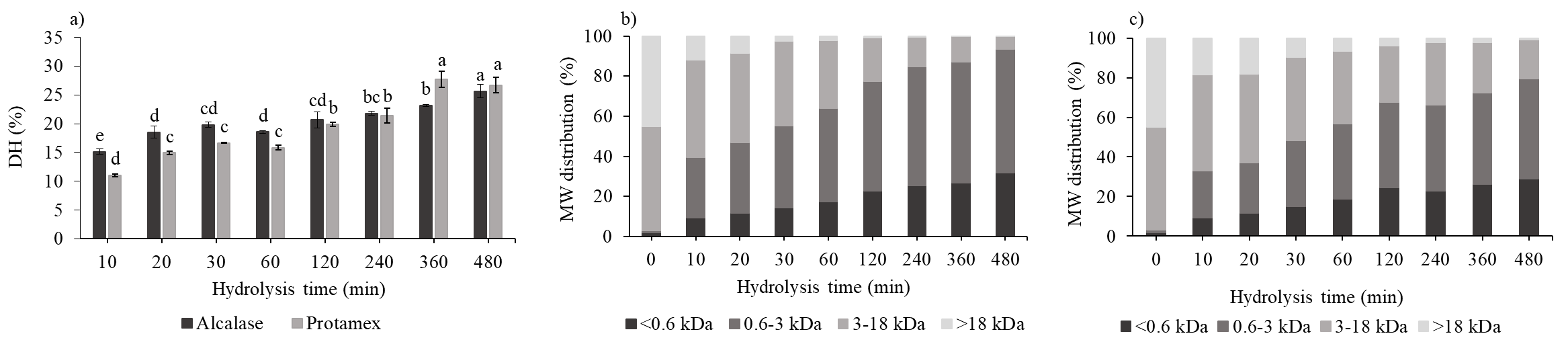
(A2) the **assessment of whey protein hydrolysates’ technological properties**, namely solubility, emulsifying, foaming and gelling properties, and *in vitro* **biological** property (antioxidant capacity).

**2. Materials and Methods**

Whey protein hydrolysates (WPHs) were obtained from a whey protein isolate (WPI) dissolved in potassium-phosphate through a hydrolysis process conducted with the use of Alcalase 2.4L or Protamex enzymes for different times, from 10 to 480 minutes, at 50 and 55°C respectively. WPHs were freeze-dried and further characterized in terms of the degree of hydrolysis, with the o-phthaldialdheyde (OPA) assay (Donkor et al., 2007), whereas the percentage molecular mass (MW) distribution was determined through HPLC analysis using a TSKgel column (Cui et al., 2022). Peptides were classified according to their MW into three groups (I group>18 kDa, II group18-3 kDa, III group 3-0.6 kDa, and IV group <0.6 kDa). Technological properties were then assessed. Solubility was performed by drying the insoluble precipitate of WPI and WPHs, solubilized in water, in a vacuum oven overnight. The foaming ability and stability indices were determined by placing 10 mL of WPI and WPHs samples, overnight solubilized in distilled water (1%), in a graduated cylinder, generating foam with an Ultraturrax® at 800 x g for 3 min and measuring it over 1 h. Similarly, WPI and WPH solutions were assayed for emulsion ability and stability with the addition of sunflower oil. After homogenization with Ultraturrax® at 1000 x g the emulsions were spectrophotometrically analysed, and the indices were calculated by the equations reported by Alonso-Miravalles et al. (2019). The gelling property was determined according to Zhao et al. (2017). WPI and WPHs were solubilized in distilled water at 5, 10, and 20% protein concentration, heated at 90 °C for 30 min, cooled at 4 °C and tested for viscoelastic properties using a rheometer (ThermoScientific RheoStress, Haake, Germany). Regarding biological properties, WPHs were tested for DPPH scavenging activity. Statistical analysis was performed by analysis of variance (one-way ANOVA) and Tukey’s comparison test.

**3. Results**

WPHs showed an increase in the degree of hydrolysis (DH) as the time of hydrolysis increased (Figure 1a). After 480 min, WPHs obtained with Alcalase and Protamex enzymes reached a comparable DH (25.65% and 26.71%, respectively). The DH increasing trend, however, depicted a statistical difference after the first 10 min of hydrolysis since Alcalase demonstrated a faster proteolytic activity as compared to Protamex. This observation was also confirmed by the hydrolysates' percentage molecular weight (MW) distribution (Figures 1b and 1c).



***Figure 1*** *Degree of hydrolysis (DH) (a) and molecular mass (MW) distribution of Alcalase (b) and Protamex (c) WPHs.*

*a-e: for each enzyme, means indicated by different letters are statistically different (p<0.05).*

Technological properties were then evaluated. Table 1 reports the solubility, foaming and emulsifying ability indices (FAI and EAI) of WPHs. The increase in hydrolysis time revealed an increase in solubility just after 10 min, when hydrolysis was conducted with both enzymes, as compared to WPI. A difference was observed in Alcalase-WPHs that, after 60 min hydrolysis, demonstrated a slight solubility reduction whereas the solubility of Protamex-WPHs maintained constant values throughout the entire hydrolysis time. An increased solubility is the result of a favoured exposure of hydrophilic groups on the surface that could enhance WPHs-water interaction. Foaming properties were thus analysed and as expected, FAI of WPHs was higher compared to WPI. Again, hydrolysis conducted with Alcalase promoted an increase in FAI from 10 to 30 min hydrolysis, whereas Protamex-WPHs exhibited a progressive FAI increase upon hydrolysis. Foaming stability (FSI) was detected to gradually increase, regardless of the enzyme used. Both solubility and foaming ability results could be mostly attributed to the variation in MW distribution (Figures 1b and 1c). The initial production of fractions with lower MW than native proteins during the first minutes of hydrolysis could have led to peptides being more prone to displace themselves at the water-air interface. However, this behaviour caused a decrease in the EAI and the emulsifying stability index (ESI) of WPHs, compared to WPI, due to the lower ability of short chains in reducing the interfacial tension. Alcalase WPHs were not able to form a gel network, while Protamex WPHs formed a gel at 10-, 20- and 30-min hydrolysis. Such results could be again attributed to the different trends in peptide dimensions obtained by the two enzymes. Finally, the *in vitro* antioxidant activity displayed a similar increasing trend until 120 min for Alcalase (88.11% ± 0.77) and 60 min Protamex hydrolysis (80.65% ± 0.33). By further increasing hydrolysis time, Protamex-obtained WPH’s antioxidant activity remained unchanged, whereas Alcalase-WPHs showed a decrease in antioxidant activity to 48.85% ± 1.92. However, WPHs were demonstrated to enhance the antioxidant activity of WPI (26.33% ± 2.18). Such results highlight the possibility to obtain, through the selection of appropriate enzyme and hydrolysis degree, the best tailored WPH-based ingredients in terms of technological and/or biological properties.

***Table 1*** *Solubility, foaming ability index (FAI) and emulsifying ability index (EAI) of WPHs.*

|  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- |
| Hydrolysis time (min) | Alcalase | | |  | Protamex | | |
| Solubility (%) | FAI (%) | EAI (m2/g) |  | Solubility (%) | FAI (%) | EAI (m2/g) |
| 0 | 88.18±0.80d | 20.4±3.9f | 2.27±0.44a |  | 88.18±0.80c | 20.4±3.9d | 2.27±0.44a |
| 10 | 92.26±0.70ab | 76.2±3.0e | 1.19±0.12bce |  | 92.20±0.91b | 72.2±2.6c | 1.82±0.43ab |
| 20 | 91.81±0.64ab | 129.6±0.0b | 1.34±0.25bcd |  | 92.59±0.87a | 100.3±10.9bc | 1.66±0.40ab |
| 30 | 93.36±0.85a | 176.5±4.9a | 1.60±0.16ab |  | 91.59±0.81ab | 104.0±5.7b | 1.05±0.22b |
| 60 | 92.48±0.52c | 139.7±3.7b | 1.50±0.09ac |  | 92.39±1.04a | 104.0±5.7b | 1.19±0.30ab |
| 120 | 90.25±0.72bc | 94.4±2.5d | 1.49±0.02bc |  | 91.83±0.95ab | 136.0±5.7a | 1.11±0.05ab |
| 240 | 90.50±0.11d | 111.7±4.2c | 0.80±0.03ce |  | 92.11±0.62a | 120.0±10.0ab | 0.97±0.02b |
| 360 | 90.32±0.48c | 106.0±2.8cd | 0.50±0.03e |  | 92.18±0.12a | 108.0±17.0ab | 1.03±0.38b |
| 480 | 90.25±0.65c | 102.2±3.1cd | 0.57±0.20de |  | 90.95±0.58ab | 122.0±19.8ab | 1.30±0.10ab |

Data show mean values (±SD, n=3). WPI data were reported for both enzymes to allow the comparison with WPHs.

ae: in the same column, means indicated by different letters are statistically different (*p*<0.05).

**4. References**

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