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Original article

The influence of pectinase treatments on the characteristics of pre-treated and lyophilised sunflower meal-whey blends

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Summary Whey and sunflower meals are primary by-products of dairy cheese and vegetable oil production, respectively. Due to their widespread abundance, their valorisation could enable the generation of various value-added food ingredients or products. In this study, an effort was made to utilise enzymatic pectinolysis along with other pre-treatments to generate mixtures from whey and sunflower meal (SFM). The increasing presence of SFM increased the critical denaturation temperature of the mixtures and induced less protein structural changes. In addition, the presence of complex carbohydrates in SFM enhanced water and oil holding capacities. In general, pectinase and ultrasonication led to enhanced foaming of the mixtures, although the foams were relatively short-lived. SFM inclusion limited foaming and emulsification capacities of the blends. In most cases, increasing the ratio of SFM to protein resulted in lower total reducing sugars (TRS), suggesting a more limited enzymatic activity. On the other hand, heating temperature or ultrasonication improved the extent of pectinolysis. Pre-treatments and pectinolysis improved the functional properties of SFM and whey protein concentrate blends and are promising as an alternative protein source in new product applications.

Keywords Pectinase, sunflower meal, ultrasonication, valorisation, whey.

Introduction

After palm, soybean and rapeseed oil, sunflower seed oil is ranked as the fourth largest vegetable oil product with an annual production of approximately 18.4 million tons in 2018 (FAO-STAT, 2019). Sunflower seeds contain on average 21 g of protein, 56.1 g of fat, 4.3 g of ash, 17.1 g of carbs and 10.3 g of fibre per 100 g, although the composition varies depending on the cultivar and growth conditions (USDA, 2021). Due to their relatively high protein content, sunflower meal or press cake streams are widely utilised as fertilisers or animal feed after oil removal. In 2021, the global sunflower seed meal production has been estimated to be approx. 23.2 million metric tons (https://www.indexmundi.com/agriculture).

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Whey, a by-product of the dairy industry produced during cheese manufacture, is a rich source of nutrients. Whey is normally rich in lactose and proteins, although its composition is variable depending on the cheese processing method and is still one of the most significant environmental pollutants of the dairy industry (Spalatelu, 2012). This is a pronounced problem especially for small-scale and/or artisanal cheese manufacturers, because of variability and extensive demands on supply chains. In Europe, the anticipated trend indicates a steady growth in the production of whey powder, with a projected increase of 250 thousand metric tons, equivalent to an 11.27% rise, in the coming years. Thus, it is estimated that the production volume will reach 2.5 million metric tons by 2031 (Shahbandeh, 2023).

Consequently, due to sustainability goals, it is crucial to direct nutritious whey streams towards

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cost-effective and sustainable methods of utilisation and generation of novel and value-added ingredients. The disposal of whey represents a significant loss of potential nutrients and energy (Zandona *et al.*, 2021), which could otherwise be utilised.

An appropriate strategy to valorise both cheese and sunflower oil processing by-product streams could entail their combination (Raak *et al.*, 2022). Various combinations of plant and animal proteins have proven successful in facilitating technologically advantageous synergies (Martínez-Bustos *et al.*, 2011; Kristensen *et al.*, 2020; Mangieri *et al.*, 2023). While oilseed press cakes are higher in fibre and polyphenols, which may affect the sensory qualities and processability, the addition of a dairy matrix containing lactose and proteins to the mix, would complement the utilisation possibilities. This investigation for the FERBLEND project ("Fermentation-induced valorization of side stream blends from oilseed and dairy industry"; https://ferblend.webspace.tu-dresden.de) might lead to various new platform products, since press cakes from other oilseeds could undergo similar research due to the potential success of sunflower seed press cakes.

High-intensity ultrasonication is a non-thermal processing technique that can be regarded as energy efficient, and environmentally friendly since utilisation of toxic chemicals or organic solvents can be avoided. Ultrasonic treatments can be administered as a pre-treatment to modify molecular structures, for example in altering of physical and functional characteristics of food proteins (Gülseren *et al.*, 2007). Thus modified proteins could potentially lead to the development of new ingredients for food manufacturing (Juodeikiene *et al.*, 2020). Ultrasonication also has the capabilities to increase the extraction yields of proteins and other bioactive components (Aiello *et al.*, 2022). These findings enhance the potential of sonication treatments in Whey-SFM blends (Dabbour *et al.*, 2018, 2021; Meng *et al.*, 2021). Conventional heating treatments can be utilised along with sonication to structure the mixtures and maintain food safety for the end-products. Enzymatic treatments are also often utilised in fibre-rich products, by addition of cell-wall-digesting enzymes. As cell walls are broken down during pectinase treatments, valuable compounds may be released (Granato *et al.*, 2022), and pectinase or other carbohydrases may enhance protein solubility via modifications of the cellular architecture or through formation of complex coacervates with improved electrostatic stabilisation (Rommi *et al.*, 2015). Therefore, this study examined the influence of pectinase treatments on heated, ultrasonicated or thermosonicated (i.e., heated and sonicated) on the properties of a dried (lyophilised) whey protein concentrate-sunflower meal (SFM) blends.

Materials and methods

Materials

Industrial sunflower meal (~420 g protein kg^{-1}) and whey protein concentrate (WPC) (~800 g protein kg^{-1}) were kindly supplied by Reka (İstanbul, Turkey) and Arla Foods amba (Viby, Denmark), respectively. WPC was utilised in order to limit the lactose content of the mixtures. Pectinase from *Aspergillus niger* was purchased from Sigma-Aldrich (P4716, Schnelldorf, Germany). All other reagents and chemicals were of analytical grade and were acquired from Sigma-Aldrich.

Preparation of freeze-dried blends

Whey protein concentrate (60 g L^{-1} wet basis) was reconstituted to a final volume of 1 L in water and, immediately afterwards, blended with varying amounts of industrial SFM samples (0, 50, 100, or 150 g L^{-1}). The mixtures were homogenised at 12 000 rpm for 3 min using an Ultra-Turrax T18 basic shear mixer (IKA, Germany).

In the following stage, the blends were subjected to one of three different treatments. Heat treatment (15 min, 85 °C) was applied using a water bath (SV 14/22, Memmert, Schwabach, Germany). Ultrasonication for 15 min was conducted using a Hielscher UP200 Ht sonicator (Hielscher Ultrasonics, Brandenburg, Germany) adjusted to its maximum power of (2000 W L^{-1} ultrasonic intensity). During the ultrasonication process, a water bath system (20 °C) was set up to immerse the sample holders and prevent the temperature rise in samples. The sample temperatures were periodically monitored via a thermometer to ensure the samples were always ≤25 °C. Additionally, samples were treated with a combined thermosonication (i.e., ultrasonication and heating, respectively) treatment, simultaneously for 15 min while heating.

The enzymatic hydrolysis of pectic polysaccharides by pectinase was carried out at pH 5 and 45 °C for 120 min. Different enzyme to substrate (SFM) concentrations were tested using 0.5 g L^{-1} of enzyme (5 U mg^{-1} protein) and 0, 50, 100 and 150 g L^{-1} of SFM, respectively. All suspensions were then heated until reaching a temperature of 85 °C and then kept for 5 min to inactivate the pectinase enzyme. Afterwards, all samples were dried using a freeze-dryer (TRS 2/2 V, Teknosem, İstanbul, Türkiye) and kept at −20 °C until further use. The samples were coded as H (heating), U (ultrasonication) and T (thermosonication) based on the treatments and followed by a number to indicate the concentration of SFM in the blend.

DNS (3,5-Dinitrosalicylic acid) assay for the determination of total reducing sugars (TRS)

Although pectinase activity may not be fully monitored by only measuring the total reducing sugars, as its reaction would result in shorter pectin chains, as a simpler test, the TRS content of the samples was determined to evaluate their presence, as well as the potential differences in pectinolysis between the samples. The assay, based on the method of Hu *et al.* (2008), is based on the colour change of the DNS reagent due to its reaction with reducing sugars. The DNS reagent was prepared by mixing 6.3 g DNS in 262 mL 2 M sodium hydroxide solution with 500 mL 185 g potassium sodium tartrate solution. After adding 5 g phenol and 5 g sodium sulphite, the solution was mixed well and cooled to the ambient temperature (25 ± 1 °C) and completed with distilled water to a volume of 1 L. Galacturonic acid (10 mM) was used as a standard solution and calibration curve was based on 1–8 mM sugar solutions. A dispersion of freeze-dried blends (1 mL) was mixed with 1.5 mL DNS reagent in test tubes and incubated at 95 °C for 5 min. The absorbance of the mixture was measured at 540 nm after being cooled to ambient temperature.

Differential scanning calorimetry (DSC)

DSC analysis was conducted according to the method of Malik *et al.* (2017) with slight modifications. Approx. 10–15 mg of sample was weighed in an aluminium pan and sealed hermetically. Pans were then heated between 20 and 150 °C at a rate of 10 °C min^{-1} using a DSC 60 plus Instrument (Shimadzu, Kyoto, Japan) to determine the thermal characteristics of the lyophilisates, i.e., peak denaturation temperature (T_d) and denaturation enthalpy (ΔH).

FT-IR spectroscopy

FT-IR analysis was conducted according to the method of He *et al.* (2018) using an IRTracer-100 FT-IR spectrometer (Shimadzu). The spectra were acquired in the range of 600–4000 cm^{-1} for the lyophilised samples at ambient temperature.

Water and oil holding capacity analysis

Water and oil holding capacities (WHC and OHC) of the lyophilised blends were determined according to the method of Aydemir & Yemenicioglu (2013). Briefly, 50 mg of dried blends and 1.5 mL of distilled water or commercial sunflower oil were stirred at ambient temperature for 20 s. After mixing, the tubes were incubated at 30 °C for 30 min and subsequently centrifuged at 15 000 ***g*** for 20 min. WBC and OBC were expressed as g of water or oil absorbed per g of dried blends, respectively.

Foaming capacity (FC) and stability (FS)

Foaming capacity (FC) and stability (FS) measurements were performed according to method of Ghribi *et al.* (2015) with slight modifications. Briefly, 25 mL of 30 g kg^{-1} lyophilisate dispersions were prepared. The solutions were then homogenised at 11 000 rpm for 3 min by using an Ultra-Turrax T18 basic shear mixer (IKA, Germany). FC was expressed as the % volume increase during foaming, and FS was determined as the % volume change during the storage procedure (0–30 min).

Emulsion activity (EA) and emulsion stability (ES)

Emulsifying activity (EA) and emulsion stability (ES) of the lyophilised blends were determined following the method of Malik *et al.* (2017). Five millilitre of 2% freeze-dried blend-bearing dispersions were homogenised with 5 mL soybean oil. Afterwards, the emulsions were centrifuged at 3000 ***g*** for 5 min. The EA of samples was calculated as the % of emulsified layer, whereas ES values accounted for the same % value, after heating the emulsions at 80 °C for 30 min and centrifuging under identical conditions as before.

Determination of total phenolic content and antioxidant activity

The technique detailed by Capanoglu *et al.* (2008) was used to extract samples for the assessment of the total phenolic content and the antioxidant activity. Each 1 g sample was mixed with 5 mL of 75% methanol bearing 0.1% aqueous formic acid solution and sonicated for 15 min (200 W). The supernatants were collected after centrifugation. The extraction procedure was carried out once again after adding an additional 5 mL of 75% methanol to the sediment. Both supernatants were pooled in a different test tube, and the final volume was set at 10 mL. Total phenolic content (TPC) measurements were conducted based on the method of Singleton & Rossi (1965). The results were reported as mg of gallic acid equivalent (GAE) per 100 g of dry weight. Total antioxidant capacity was conducted by two different methods including 2-diphenyl-1-picrylhydrazyl (DPPH) assay and Copper (II) reducing antioxidant capacity (CUPRAC) methods in accordance with Kumaran (2006) and Apak *et al.* (2004), respectively. The results were reported in mg Trolox equivalency (TE) per 100 g of dry weight.

Statistical analysis

At least three replicates were utilised for all analyses. The results were presented as sample means ± standard deviations. Analysis of variance (ANOVA) was used in order to determine whether there was a significant difference between the treatments ($P < 0.05$). Duncan's multiple tests were performed ($P < 0.05$) using IBM SPSS Statistics 27.0 (SPSS Inc., Chicago, IL, USA).

Results and discussion

Total reducing sugar (TRS) content

Polygalacturonanases including certain pectinases hydrolyse the α-1,4-glycosidic bond in polygalacturonic acid chains, leading to an increase in reducing-sugar groups. Consequently, the action of pectinases can be evaluated via TRS concentration (Couri *et al.*, 2013). With an increase in the concentration of SFM in the WPC-SFM mixtures, the total amount of reducing sugars decreased, regardless of pretreatments and/or enzyme applications (Fig. 1). The reduction in free sugars may be in part due to their reaction with the protein present in the mixture. However, both H- and U-treated samples with pectinase underwent enzymatic hydrolysis, as demonstrated by the higher TRS contents (Fig. 1). The extent of reducing sugars released by pectinase treatments was limited, compared to dilution effect generated by the addition of SFM, since a significant amount reducing sugars are also present in whey protein concentrate samples. As expected, due to their ability to release some of the long-chain carbohydrates from the cell wall, heating or ultrasonication treatments enhanced the pectinolysis of the mixtures, and some enhancement in TRS content was observed after these treatments compared to non-enzyme-treated samples.

DSC analysis

DSC was used to determine the thermal transitions within the mixtures, which were measured as an average denaturation temperature (T_d) and enthalpy (ΔH) of powdered mixtures of SFM and WPC. In all cases, the samples demonstrated a monomodal and broad denaturation peak. Increasing the SFM concentration in the pectinase-treated and ultrasonicated samples shifted the T_d and ΔH values (Fig. 2A), suggesting that the energy required to denature or unfold a protein structure was lower. Since SFM samples were press cakes of industrial origin, prior to the current investigations, their partial denaturation was expected. The thermal transition of proteins (i.e., denaturation) shifted to higher temperatures for non-enzyme-treated samples in the presence of higher SFM concentrations.

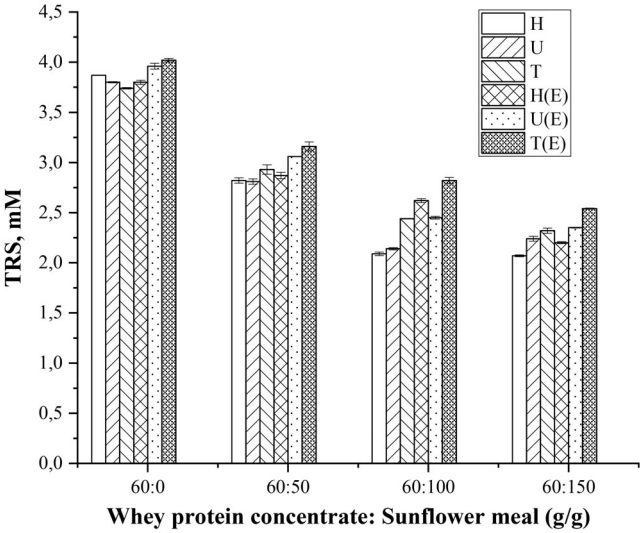

Figure 1 Total reducing sugar (TRS) concentration of lyophilised blends as a function of sunflower meal (SFM) concentration (E, enzyme-treated; H, heating; U, ultrasonication; T, thermosonication).

It is important to note that WPC powders show high T_d values (approx. 159.4 °C) than SFM proteins (approx. 85 °C), according to literature (Briones-Martínez *et al.*, 1997). It was concluded that the blends underwent partial structural changes after ultrasonication, altering the thermal behaviour. In other words, the reduction in enthalpy indicated that ultrasonicated blends were at least partially unfolded prior to enzymatic treatments, which significantly lowered the denaturation enthalpy of proteins (Gülseren *et al.*, 2007; Malik *et al.*, 2017). On the other hand, the denaturation peak shifted to higher temperatures in the presence of SFM for both heat and ultrasound-treated samples (Fig. 2B). Consequently, SFM increased the thermal stability of the blends, whereas ultrasonication pre-treatments lowered stability. The decrease in T_d, especially after ultrasonic and thermosonication treatments for enzyme-treated samples confirmed the weakening of protein–protein bonds (Table S1). In the recent literature, ΔH increased when extended ultrasonic treatments were administered during protein extraction, possibly due to protein aggregation (Sert *et al.*, 2022), where shorter durations also lowered the enthalpy values.

FT-IR analysis

The FT-IR spectra of the lyophilised blends were recorded to monitor the differences induced by heating, ultrasonication, and/or pectinase treatments. Only Amide I (1644 cm^{-1}) regions were taken into consideration to study the protein structure of the samples, as

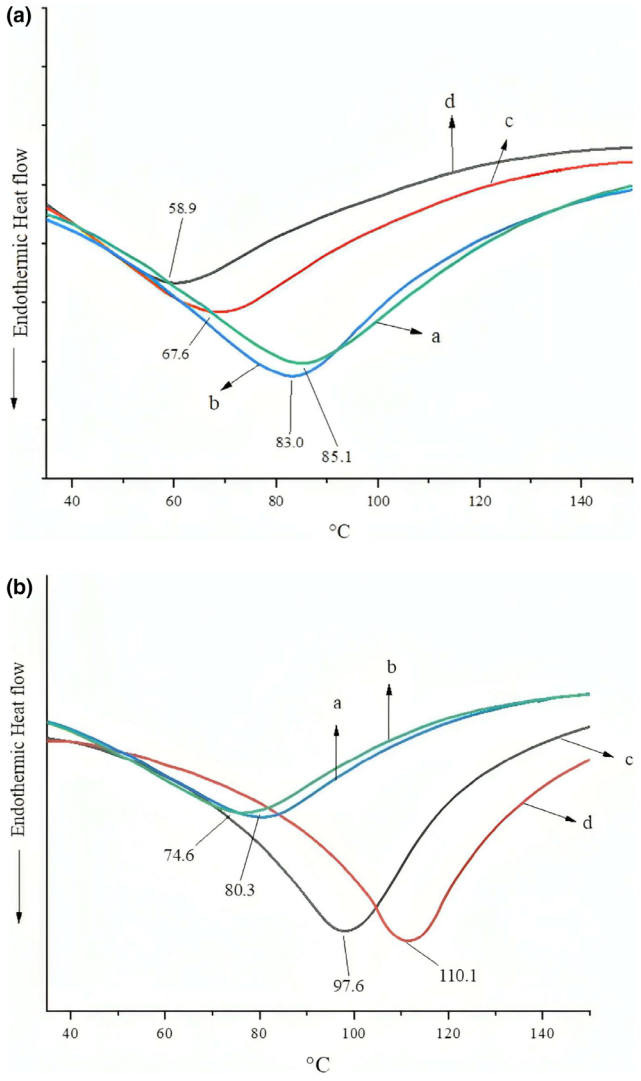

Figure 2 (a) DSC thermograms for both pectinase and ultrasound-treated lyophilised blends (a: 0% SFM; b: 5% SFM; c: 10% SFM; d: 15% SFM). (b) DSC thermograms of both heat- and ultrasound-treated lyophilised blends (a: 0% SFM; b: enzyme-treated 0% SFM; c: 5% SFM; d: enzyme-treated 5% SFM).

they are related to random coil structures (Carbonaro & Nucara, 2010). The fingerprint region of disaccharides (1077 cm^{-1}) and the range of 1600–1700 cm^{-1} representing the Amide I region was more distinct for samples not containing SFM (Fig. 3). Intense bands around 1644 cm^{-1} were observed for all samples, indicating random coil conformation. A change of intensity in the Amide-I region (the stretching vibration of C=O, the bending of in-plane N-H, and the stretching modes of C-N) due to the concentration sunflower meal and treatments could prove a modification of secondary structural elements because of the micro-streaming and cavitational forces (Zhao *et al.*, 2021). The peak position in the Amide-I region changed after ultrasonication and heat treatments, indicating that the secondary structure of both WPC and sunflower proteins were partly altered (Malik & Saini, 2018), where whey proteins were influenced more significantly potentially due to the changes in sulphur-containing amino acids (Gülseren *et al.*, 2007). The shear forces, shock waves, and turbulence produced by ultrasonication may be the cause of the changes in the protein secondary structures, since cavitation alters the secondary structures of the protein molecules by interfering with interactions between the local sequences of amino acids and other components of the protein molecules. Furthermore, ultrasonic exposure may result in selective hydrogen bond rupture, possibly leading to the increase in disordered secondary structures (Aiello *et al.*, 2022). As demonstrated in TRS content analyses, heating or ultrasonication treatments enhanced the pectinolysis of the mixtures, which might have also influenced structural elements in protein molecules.

Water- and oil-holding capacity (WHC/OHC)

WHC and OHC, expressed as the amount of water or oil held by 1 g of lyophilised blends, respectively, ranged between 2.4 to 7.1 g water g^{-1} and 5 to 9.2 g oil g^{-1} for non-enzyme-treated samples (Table 1). On the other hand, for enzyme-treated samples, these values varied between 3.7 to 7.9 g water g^{-1} and 5.9 to 7.7 g oil g^{-1}, respectively. The ultrasonicated blends had lower WHC and OHC when compared with the heat-treated counterparts. This might be explained by the sonication-induced partial denaturation of proteins and subsequent exposure of hydrophobic groups, which led to a decrease in WHC. Previously, enhanced surface hydrophobicity was observed in sonicated proteins, however compared to the previous literature the influence of sonication on surface hydrophobicity was regarded as moderate, since thermal denaturation increases hydrophobicity more substantially (Gülseren *et al.*, 2007).

In the thermally denatured samples, protein aggregation led to changes in WHC or OHC. Since the conformational alterations in protein molecules and the modifications in surface hydrophobicity following ultrasonic treatments could lead to the formation of more soluble protein aggregates, it is plausible that changes in the hydrophobic surface area are responsible for a decrease in WHC compared to non-sonicated counterparts (Malik *et al.*, 2017; Sert *et al.*, 2022). Furthermore, as the added SFM concentrations increased, WHC and OHC values increased, regardless of pretreatments or enzyme applications. The concentration of polysaccharides (i.e., fibre) also increased with SFM

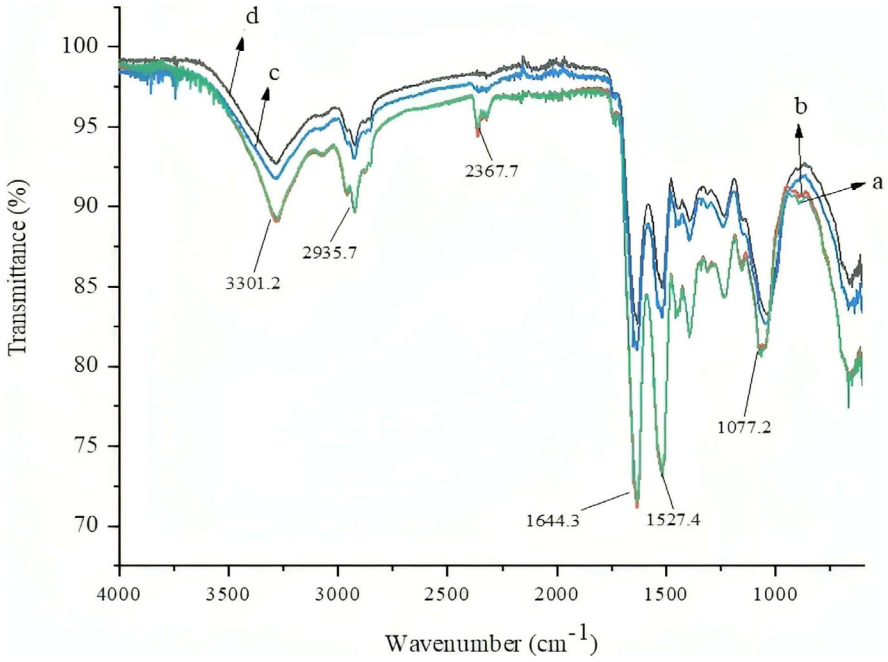

Figure 3 FT-IR spectra of both heat- and ultrasound-treated lyophilised blends (a: 0% SFM; b: enzyme-treated 0% SFM; c: 10% SFM; d: enzyme-treated 10% SFM).

content, which possibly affected WHC and OHC values favourably (Figure S1A and B).

Foaming capacity (FC) and stability (FS)

Molecular flexibility, capacity to reduce surface tension, and maintain air bubbles at the gas–liquid surface by slowing down the coalescence of bubbles are all linked to foaming qualities of proteins (Boye *et al*., 2010; Ghribi *et al*., 2015). FS refers to the ability to hold air bubbles for a predetermined time. In contrast, FC refers to the relative volume increase in a foam caused by the confinement of air bubbles. FC and FS values are listed in Table 1. FC values ranged from approx. 12.5% to 125% for non-enzyme-treated and from 50% to 125% for enzyme-treated samples. The FC and FS values of blends were improved after ultrasound treatments compared to heat-treated samples (Table 1). While the stability of foams prepared using the heat-treated samples was relatively low, ultrasonication partly enhanced the foam stability by up to approx. 2 h. The ultrasonicated sample with no SFM (U0%) demonstrated the highest FC and FS values for pectinase-treated and non-treated samples. Ultrasonic treatments potentially facilitated the production of foams with enhanced stability compared to heat treatment. Previously, ultrasonication treatments have been linked to enhanced surface activity at the air-water interface (Gülseren *et al*., 2007), which is coherent with the current findings.

The foaming capacity of proteins depends largely on their diffusivity at the gas–liquid interface, and an increase in FC may be related to the fact that ultrasound enhances surface activity and dispersibility of proteins. In addition, ultrasonic treatment enhanced the foam stability more significantly than the heat-treated samples (Table 1). With ultrasonication, the molecular flexibility was improved, lowered the surface tension, and facilitated the creation of strong elastic films around dispersed gas bubbles (Sharma *et al*., 2010).

While the FC and FS of blends were poor, the foaming performances were slightly but significantly improved with pectinase treatments. The presence of low-molecular-weight oligosaccharides was previously shown to enhance the surface activity of aqueous protein dispersions (Guzey *et al*., 2003; He *et al*., 2021). The FC and FS of samples decreased as SFM content increased, since the foaming properties of the meal were inferior, foam structure was fragile, and foam stability at room temperature was low, as was also reported by Sogi *et al*. (2002) for tomato seed meal. In addition, the solubility of powders plays a crucial role in foam properties. Sunflower meals and isolates exhibit higher foaming capacity at pH 3.0–5.0, while demonstrating improved foaming stability at pH 7–9.

Table 1 Water and oil-binding capacities, foaming, and emulsifying properties of lyophilised blends

	WHC (g g^{-1})	OHC (g g^{-1})	EC (%)	ES (%)	FC (%)	FS 10 min (%)	FS 30 min (%)
Non-enzyme-treated samples							
H 0%	4.1 ± 0.1^{def}	6.2 ± 0.2^{bc}	62.5 ± 3.5^{ab}	67.5 ± 3.5^{ab}	50 ± 2	12.5 ± 1	0 ± 0
H 5%	5.1 ± 0.2^{bcde}	6.7 ± 0.0^{abc}	46.3 ± 1.8^{cde}	51.3 ± 1.8^{cde}	12.5 ± 1	5 ± 0	2.5 ± 0
H 10%	5.3 ± 0.1^{bcd}	7.3 ± 0.4^{abc}	43.8 ± 1.8^{de}	48.8 ± 1.8^{de}	12.5 ± 1	5 ± 0	0 ± 0
H 15%	6.4 ± 0.2^{abc}	9.2 ± 0.4^{a}	36.3 ± 1.8^{e}	41.3 ± 1.8^{e}	12.5 ± 1	5 ± 0	5 ± 0
U 0%	2.4 ± 0.1^{f}	7.1 ± 0.5^{abc}	72.5 ± 3.5^{a}	77.5 ± 3.5^{a}	125 ± 5	90 ± 7	37.5 ± 3
U 5%	3.5 ± 0.1^{ef}	5.0 ± 0.1^{c}	62.5 ± 3.5^{ab}	67.5 ± 3.5^{ab}	87.5 ± 6	25 ± 1	0 ± 0
U 10%	5.7 ± 0.2^{abcd}	6.9 ± 0.1^{abc}	57.5 ± 3.5^{bc}	62.5 ± 3.5^{bc}	25 ± 3	12.5 ± 0	0 ± 0
U 15%	5.9 ± 0.4^{abc}	7.4 ± 0.1^{abc}	52.5 ± 3.5^{bcd}	57.5 ± 3.5^{bcd}	37.5 ± 2	12.5 ± 1	12.5 ± 1
T 0%	3.6 ± 0.1^{ef}	6.1 ± 0.4^{bc}	62.5 ± 3.5^{ab}	67.5 ± 3.5^{ab}	100 ± 4	40 ± 3	12.5 ± 0
T 5%	5.0 ± 0.1^{cde}	7.9 ± 0.4^{ab}	47.5 ± 3.5^{cde}	52.5 ± 3.5^{cde}	87.5 ± 5	45 ± 2	12.5 ± 0
T 10%	6.8 ± 0.0^{ab}	7.9 ± 0.7^{ab}	60.0 ± 0.0^{b}	65.0 ± 0.0^{b}	42.5 ± 3	25 ± 2	12.5 ± 1
T 15%	7.1 ± 0.5^{a}	5.7 ± 0.1^{bc}	62.5 ± 3.5^{ab}	67.5 ± 3.5^{ab}	50 ± 4	32.5 ± 1	12.5 ± 1
Pectinase-treated samples							
H 0%	4.0 ± 0.5^{de}	6.4 ± 0.2^{a}	52.5 ± 3.5^{bcd}	57.5 ± 3.5^{bcd}	87.5 ± 7	12.5 ± 0	0 ± 0
H 5%	$4.4 \pm 0.2b^{cde}$	6.3 ± 0.4^{a}	47.5 ± 3.5^{cd}	52.5 ± 3.5^{cd}	62.5 ± 5	25 ± 1	0 ± 0
H 10%	6.2 ± 0.1^{abcd}	6.4 ± 0.2^{a}	41.3 ± 1.8^{ef}	46.3 ± 1.8^{de}	62.5 ± 5	25 ± 2	0 ± 0
H 15%	7.8 ± 0.5^{a}	7.0 ± 0.0^{a}	32.5 ± 3.5^{f}	37.5 ± 3.5^{de}	50 ± 4	25 ± 1	12.5 ± 0
U 0%	3.7 ± 0.0^{e}	5.9 ± 0.2^{a}	71.3 ± 1.8^{a}	76.3 ± 1.8^{a}	125 ± 9	100 ± 6	25 ± 1
U 5%	4.1 ± 0.0^{cde}	7.0 ± 0.0^{a}	62.5 ± 3.5^{ab}	67.5 ± 3.5^{ab}	75 ± 7	30 ± 2	5 ± 0
U 10%	6.3 ± 0.0^{ab}	6.8 ± 0.0^{a}	57.5 ± 3.5^{abc}	62.5 ± 3.5^{abc}	75 ± 3	25 ± 1	12.5 ± 1
U 15%	6.3 ± 0.1^{abc}	7.6 ± 0.4^{a}	52.5 ± 3.5^{bcd}	57.5 ± 3.5^{bcd}	75 ± 2	37.5 ± 2	25 ± 2
T 0%	4.0 ± 0.0^{cde}	6.7 ± 0.4^{a}	55.0 ± 7.1^{bcd}	60.0 ± 7.1^{bcd}	112.5 ± 8	50 ± 3	25 ± 1
T 5%	5.2 ± 0.4^{bcde}	6.4 ± 0.2^{a}	52.5 ± 3.5^{bcd}	57.5 ± 3.5^{bcd}	100 ± 6	55 ± 4	20 ± 1
T 10%	6.5 ± 0.0^{ab}	7.0 ± 0.3^{a}	42.5 ± 3.5^{de}	47.5 ± 3.5^{de}	62.5 ± 3	25 ± 2	0 ± 0
T 15%	7.9 ± 0.6^{a}	7.7 ± 0.1^{a}	52.5 ± 3.5^{bcd}	57.5 ± 3.5^{bcd}	55 ± 4	37.5 ± 1	12.5 ± 1

Data represent a mean ± SD ($n = 2$). Mean values with the same letter within the same column and the same enzyme treatment are not significantly different.

EC, Emulsion capacity; ESI, Emulsion stability; FC, Foaming capacity; FS, Foam stability; H, Heating; OBC, Oil-binding capacity; T, thermosonication; U, Ultrasonication; WBC, Water-binding capacity.

These results highlight the substantial impact of protein solubility on sunflower protein foaming properties (Salgado *et al.*, 2012; Dabbour *et al.*, 2018).

Emulsion formation capabilities

Emulsifying activity (EA) and emulsion stability (ES) were used to assess the emulsifying abilities of the blends. EA is the ability of emulsifiers to be adsorbed at the interface of water and oil, while ES represents their capacity to be retained at the oil–water interface during storage procedures. As seen in Table 1, the EA and ES values of the ultrasonicated samples were higher than the heat-treated samples for both pectinase-treated and non-treated samples, possibly due to increased molecular flexibility and/or surface hydrophobicity of the protein molecules in the mix. As increasing amounts of SFM were added to the samples, EA and ES values decreased regardless of the pretreatment or enzyme applications (Table 1). In most cases, emulsions with limited stability were generated, since rapid phase separation (approx. 10 min) took place (data not shown). Since sunflower meal proteins have low solubility, increasing their concentration in the blend will likely have a negative impact on solubility, EA, and ES. As in the pH range of the prepared blends (around pH 6.4), the minimal solubility (40%) was found at pH 5–7 in the literature (Pickardt *et al.*, 2015; Slabi *et al.*, 2020).

Effect of pectinase and heating treatments on total phenolic content and total antioxidant capacity

The total phenolic contents (TPC) and total antioxidant capacities of the samples were presented in Tables 2 and 3 for non-enzyme-treated and pectinase-treated samples, respectively. The TPC of non-enzyme-treated vs. pectinase-treated samples varied from 4.2 to 47.9 mg GAE/100 g and 4.4 to 59.5 mg GAE/100 g, respectively. In general, increasing the SFM content resulted in a significant increase in the TPC of all samples ($P < 0.05$). In addition, the CUPRAC and DPPH assays were used to examine the antioxidant capacity of the samples. The total

Table 2 Total phenolic content and antioxidant activity of non-enzyme-treated samples

Analysis					
TPC (GAE, mg/100 g)					
H 0%	4.2 ± 0.0d	U 0%	5.3 ± 0.0d	T 0%	5.0 ± 0.0c
H 5%	27.0 ± 2.6c	U 5%	20.7 ± 1.3c	T 5%	33.5 ± 1.6b
H 10%	36.2 ± 0.8b	U 10%	33.7 ± 0.4b	T 10%	44.9 ± 6.3ab
H 15%	47.2 ± 4.2a	U 15%	45.0 ± 1.5a	T 15%	47.9 ± 1.6a
CUPRAC (TE, mg/100 g)					
H 0%	7.3 ± 0.1d	U 0%	7.3 ± 0.0c	T 0%	6.8 ± 0.0c
H 5%	81.3 ± 0.4c	U 5%	68.8 ± 1.7b	T 5%	96.6 ± 0.6b
H 10%	117.6 ± 0.8b	U 10%	111.3 ± 1.9ab	T 10%	130.9 ± 4.3ab
H 15%	141.9 ± 12.8a	U 15%	136.2 ± 2.4a	T 15%	146.4 ± 8.4a
DPPH (TE mg/100 g)					
H 0%	4.8 ± 0.1c	U 0%	6.0 ± 0.3c	T 0%	5.9 ± 0.2c
H 5%	28.2 ± 0.7b	U 5%	24.9 ± 0.2b	T 5%	31.1 ± 0.2b
H 10%	120.2 ± 0.5a	U 10%	95.1 ± 1.4ab	T 10%	110.0 ± 2.6ab
H 15%	119.8 ± 0.9a	U 15%	109.9 ± 5.6a	T 15%	117.5 ± 3.3a

Values are mean ± SD ($n = 3$). Means with different superscript in a column differ significantly ($P < 0.05$).
H, heating; T, thermosonication; TPC, total phenolic content; U, ultrasonication.

Table 3 Total phenolic content and antioxidant activity of pectinase-treated samples

Analysis					
TPC (GAE mg/100 g)					
H 0%	5.1 ± 0.0d	U 0%	4.4 ± 0.1c	T 0%	5.1 ± 0.2c
H 5%	29.2 ± 1.1c	U 5%	13.4 ± 0.2b	T 5%	27.2 ± 3.2b
H 10%	53.2 ± 1.0a	U 10%	42.9 ± 0.5ab	T 10%	53.8 ± 3.3ab
H 15%	43.6 ± 1.1b	U 15%	49.5 ± 4.3a	T 15%	59.5 ± 0.0a
CUPRAC (TE mg/100 g)					
H 0%	5.7 ± 0.1c	U 0%	6.7 ± 0.0c	T 0%	5.8 ± 0.1c
H 5%	80.0 ± 2.0b	U 5%	76.2 ± 4.3b	T 5%	79.1 ± 3.7b
H 10%	138.2 ± 8.6ab	U 10%	153.1 ± 5.7a	T 10%	154.0 ± 6.5ab
H 15%	143.0 ± 0.6a	U 15%	139.1 ± 3.0ab	T 15%	137.0 ± 6.1a
DPPH (TE mg/100 g)					
H 0%	3.6 ± 0.2c	U 0%	3.8 ± 0.5c	T 0%	4.9 ± 0.0c
H 5%	28.2 ± 0.1b	U 5%	28.3 ± 0.2b	T 5%	27.5 ± 0.2b
H 10%	117.4 ± 0.5a	U 10%	119.2 ± 0.9ab	T 10%	115.0 ± 4.1a
H 15%	118.4 ± 0.5a	U 15%	126.3 ± 3.0a	T 15%	119.5 ± 0.7a

Values are mean ± SD ($n = 3$). Means with different superscript in a column differ significantly ($P < 0.05$).
H, heating; T, thermosonication; TPC, total phenolic content; U, ultrasonication.

antioxidant capacities of samples significantly increased with the SFM content. As shown in Tables 2 and 3, the addition of SFM increased antioxidant activity compared to the controls by 18.6–25.1 and 18.3–33.2 times, respectively, based on the CUPRAC and DPPH methods. Since the sunflower meal is rich in polyphenols (2–5%), its utilisation in chemical, pharmaceutical and food applications is promising (Náthia-Neves & Alonso, 2021). Table 3 findings pointed out in some of the current experiments, pectinase and other treatments were observed to enhance the antioxidative activity of SFM phenolics. Consequently, the current treatments may be instrumental in mixing of these two major by-product streams under controlled circumstances.

Conclusion

Combining by-products has significant potential for creating environmentally friendly and appealing food products or ingredients while also minimising food wastage. Whey and sunflower meals, derived from dairy cheese and vegetable oil production, respectively, are key by-products. Leveraging their abundant availability could facilitate the creation of value-added food ingredients or products. This study aimed to use

enzymatic pectinolysis and other pre-treatments to produce blends from whey protein concentrate and sunflower meal. The increasing presence of SFM raised the critical denaturation temperature of the blends, causing fewer changes in protein structure. Moreover, SFM's complex carbohydrates increased water and oil holding capacities. Generally, pectinase and ultrasonication enhanced the foaming of the blends, despite relatively short-lived foams. SFM inclusion constrained foaming and emulsification capacities. In most instances, a higher SFM-to-protein ratio resulted in lower total reducing sugars, indicating reduced enzymatic activity. Conversely, elevated heating temperature or ultrasonication improved pectinolysis extent. Overall, pre-treatments and pectinolysis enhanced the functional properties of SFM and whey protein concentrate blends, showing promise as an alternative protein source in new product applications.

Author contributions

Zeynep Saliha Güneş: Conceptualization; methodology; writing – original draft; formal analysis; visualization. **Norbert Raak:** Conceptualization; methodology; writing – review and editing; supervision. **Milena Corredig:** Conceptualization; writing – review and editing; supervision. **Ibrahim Gülseren:** Conceptualization; writing – original draft; writing – review and editing; supervision; resources.

Conflict of interest

The authors declare no conflict of interest.

Ethical approval

Ethics approval was not required for this research.

Peer review

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Data availability

Data will be presented upon request.

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Supporting Information

Additional Supporting Information may be found in the online version of this article:

Figure S1. (A) Colour of non-enzyme-treated lyophilised blends modulated by pretreatments and the concentration of SFM. SFM, sunflower meal; H, heating; U, ultrasonication; T, thermosonication. (B) Colour of enzyme-treated lyophilised blends modulated by pretreatments and the concentration of SFM. SFM, sunflower meal; H, heating; U, ultrasonication; T, thermosonication.

Table S1. The denaturation temperature (T_d) and enthalpy (ΔH) values of powdered mixtures of SFM and WPC.