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VALORIZATION of HAZELNUT and SESAME PROTEIN ISOLATES in SUSTAINABLE MEATBALL MANUFACTURE

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ABSTRACT

The ever-increasing global demand for proteins necessitates the generation of sustainable plant protein products. The aim of the current study is the utilization of cold press cakes for the generation of hazelnut and sesame protein isolates and their valorization in meatball manufacture. Protein isolates were generated from cold press cakes using an alkaline extraction-isoelectric precipitation (AE-IP) method. The functional properties (solubility, emulsion and foam formation capacity, and oil and water holding capacity) of hazelnut and sesame protein isolates were examined. Furthermore, physicochemical and sensory properties (texture, size, color, and sensory attributes) of the meatball samples fortified by these isolates were investigated. Protein fortification altered color of the meatballs and increased the firmness of meatballs at elevated protein contents. However, toughness or meatball size were unaffected by fortification. The differences between treatments were attributed to the molecular size characteristics of proteins and fiber content in the isolates. Sensory data confirmed that the acceptance for meatballs were maximum for samples fortified with 5% protein and sesame protein isolate was more preferable over hazelnut counterparts. Since commercial meatballs contain approx.20% protein, ≥5% plant protein fortification could be a significant development in protein content and sustainability in meatball production.

1.Introduction

The trend towards the consumption of protein-rich food products have increased over the recent decades along with the world population and a keen research interest is especially present in novel protein resources. Millions of tons of agricultural or industrial food by-products or waste are discarded every year globally. Although the majority of such streams are mostly biodegradable, their disposal leads to serious environmental problems such as water pollution and generation of unwanted odors. These by-product streams can potentially be utilized as low-cost resources of proteins in human nutrition (Ogunwolu et al., 2009). However, inclusion of novel proteins should not lead to impaired sensory, functional or nutritional properties in the final products. In this context, many research studies have been conducted on plant protein resources including canola, soy and various pulses, all of which contain significantly high amounts of protein (Moure et al., 2001).

Currently, the demand for ready-to-eat foods is also increasing all over the world. In this product category, ready-to-cook meatball varieties represent an essential group (Yılmaz, 2015). Global meat industry aims to increase

the product quality, and nutritional attributes in their products while generating safe products at reduced production costs. In this context, there is a need for protein components that have the potential to increase the protein content in ready-to-eat meatballs while sustaining the organoleptic properties of products, and reducing the overall cost. Furthermore, utilization of plant proteins in meat products could potentially lower their environmental footprint (Saget et al., 2021). The conversion efficiency of plant proteins to animal proteins is known to be in the order of 15% (Day, 2013).

In conventional oil processing, vegetable oils are generally extracted from ground seeds by treating them with organic solvents such as hexane, which is followed by solvent evaporation. Cold press technology is an alternative method during which solvent extraction or heat application procedures are not necessary (Parker et al., 2003). Consequently, this gentle approach enables the preservation of residual materials in cold press cakes. Since seeds contain significant amounts of proteins, their corresponding cold press cakes are further concentrated in proteins (Coşkun et al., 2019) and can be utilized in food products (Coşkun et al., 2020).

Hazelnuts (*Corylus avellana* L.) are highly sought after in pastries and chocolate industries due to their desirable organoleptic properties. Hazelnut components are also widely used as a flavor and aroma additive in the bakery and dairy industries, salad dressing products due to their rich protein, fat, vitamin and mineral contents (Fallico et al., 2003). The protein content in the deoiled hazelnut cakes is in the range of 35-41% (Yağcı and Göğüş, 2008). Some of the bioactive attributes of hazelnut proteins and peptides have been recently reviewed (Aydemir et al., 2014; Çağlar et al., 2021).

Sesame seeds (*Sesamum indicum* L.) have been cultivated for nearly 4000 years and are characterized with a high-energy value and fat content. Potential health benefits of sesame include anti-oxidative, anticancer, anti-hypersensitivity, and anti-immunoregulatory activities. The seeds are used in oil manufacture, salad making and various food formulations. Sesame seeds contain 50-60% oil, 18-25% protein, 13.5% carbohydrate and 5% ash. Oxidative stability of sesame oil can be attributed to endogenous antioxidant lignans along with tocopherols. Sesame seeds have long been considered in the Eastern regions of the world as a healthy food that prevents aging, and serve as a rich source of calcium (approx. 1%) and phosphorus (approx. 0.7%) (Prasad et al., 2012). Based on these data, supplementation of foods with hazelnut and sesame protein products could enhance various nutritional components of foods.

Protein molecules have the capabilities to hold oil and water and improve the structural qualities of foods. Through water holding, proteins swell and affect the rheological and textural properties of foods (Seena and Sridhar, 2005). Water and oil holding capacities of proteins are functions of shape, size, hydrophobic and hydrophilic interactions of the protein molecules. The interactions between proteins and fats/oils affect the sensory quality of many foods. Proteins with low solubility and high hydrophobicity can hold large amounts of fats and oils (Guerra et al., 2011). Consequently, the influence of protein fortification on food properties including color, texture, water and oil holding capacities, and the ability to stabilize emulsions and foams need to be well-understood.

In this study, based on appropriate aqueous extraction techniques, protein isolates were manufactured from cold press cakes of hazelnut and sesame seeds. In order to determine the suitability of these protein isolates to meatball manufacture, functional properties of the protein ingredients and final products were evaluated. In this context, solubility, foam and emulsion stabilization characteristics as well as fat and water holding capacities were investigated. Furthermore, their influence on plant protein fortified meatball characteristics including sensory, visual and textural attributes were studied in an effort to reduce the environmental footprint of meatball products and increase their protein contents. Meatballs are characterized by an approximate protein

content of 20% (Serdaroğlu et al., 2005). In the current study, both protein fortification and sustainable production of meatballs have been targeted.

2. Materials and methods

2.1. Materials

Cold press cakes were obtained under gentle processing conditions such as low processing temperatures (≤40^{0}C). Hazelnut (Mecidefendi, İzmir, Turkey) and sesame (Vitalling, Adana, Turkey) press cakes were obtained from the domestic producers of cold press oils and the cakes were stored at +4ºC until further use.

All chemicals used in the analyses were purchased from Sigma (Schnelldorf, Germany). Beef samples (M. gluteus medius), spices, bread crumbs and onion powder were used as raw materials in meatball production. The fat content of ground beef used for meatballs was approx. 15%. All materials used in meatball production were supplied from local supermarkets. Meatball production and their corresponding analyses were carried out at Tekirdağ Namık Kemal University (NKU), Turkey, Microbial Biotechnology Laboratory of Dept. of Agricultural Biotechnology and Eksun Food Company R&D Center, Tekirdağ, Turkey. Meatball texture was studied at NKU Central Research Laboratories.

2.2. Methods

2.2.1 Manufacture of protein isolates

Alkali extraction-isoelectric precipitation (AE-IP) method was applied for the production of protein isolates from cold press cakes (Coşkun et al., 2019). In order to prevent protein denaturation, solvent extraction was not administered. Firstly, the cake samples were ground down to approx. 2 mm. The ground samples were mixed with ultra-pure water (Millipore, Simplicity, USA) at a ratio of 1:15 (sample:water). The mixture was kept stirred at 500 rpm using a magnetic stirrer for 1 h. Immediately afterwards, pH value of the mixture was brought to pH 9.5. Insoluble materials were separated by centrifuging the mixture at 4200xg for 15 minutes. Isoelectric precipitation of the proteins (pH 4.5) was promoted using 1 N HCl and the mixture was centrifuged again under the same conditions as before. The precipitated portion was lyophilized (Teknosem, Toros TDS 2/2V, İstanbul, Turkey) and stored in the freezer (-20°C) until subsequent procedures. Representative freeze-dried protein isolates were shown on Figure 1.

Figure 1. Freeze dried protein isolates. HPI (left) and SPI (right).

2.2.2. Analysis of the composition and functional properties of protein isolates

Firstly, hazelnut or sesame protein solubility was determined using Sigma-Aldrich Total Protein Kit, Micro Lowry, Petterson's Modification (TP0300). Total protein content of the isolates was assayed using the Kjeldahl method (Da Silva et al., 2021). The moisture contents of hazelnut and sesame protein isolates (HPI and SPI, respectively) were

determined by the gravimetric method (Da Silva et al., 2021).

For this purpose, 3 grams of samples were weighed into the stainless steel containers and the samples were kept for 4 hours in a 105±2 ºC incubator (Memmert, unb400, Germany). When constant weight was achieved, the amount of moisture removed from the samples (%) was calculated. Ash content was analyzed after an incubation period of 8 hours at 550±15ºC (Magmatherm, mt1105, Turkey) (Da Silva et al., 2021).

The water and oil holding capacity of protein isolates were determined by adding 1 g protein isolate samples to 10 ml of pure
water or 8 ml of soy oil (Sanchez-Vioque et al., 1999). The mixture was kept vortexed (Vortex Genie 2, Scientific Industries, USA) for 30 seconds every 5 minutes for 30 minutes. Thus prepared sample was kept at room temperature and centrifuged for 30 minutes at 3000xg (Selecta, Myxtasel BL., Cham, Switzerland). After this procedure, the water or oil holding capacity was determined in terms of g absorbed water or oil /g protein.

The emulsion activity index (EAI) and emulsion stability index (ESI) values of the protein isolates were determined as presented by Zhang et al. (2021). Aqueous protein dispersions (6 ml, 0.1%) and 2 ml of soy oil (Sigma, S7381) were homogenized for 3 minutes at 4000 rpm using a shear mixer (Wisd, H6-15A, Ireland). Immediately after preparation, an aliquot (50 µl) was taken from the emulsion and diluted with a 10 ml 0.1% SDS solution. The absorbance of the diluted sample was determined at 500 nm (i.e., turbidity) (Optima, SP 3000 UV VIS, Japan) and the corresponding EAI and ESI values were calculated.

The foaming capacity and foam stability of protein isolates were measured according to the method published by Chabanon et al. (2007). Foaming capacities of samples were based on volume change (%) at the time of preparation and the stability of foams (i.e., foam volume) were monitored for 90 minutes after preparation.

2.2.3. Preparation and analysis of protein enriched meatballs

Meatball samples were prepared using 0.3% black pepper, 0.2% red pepper, 0.5% cumin, 5% onion powder, 2% salt, 7% breadcrumbs and ground meat to complete a 1 kg formulation. Hazelnut protein isolate (HPI) or sesame protein isolate (SPI) (5, 10, 15, or 20%) samples were added to a 200 g sub-sample from the 1 kg mix. The ingredients were mixed by kneading, and the mixture was portioned into round meatballs with an approximate diameter of 8 cm. The cooking process was carried out on a non-stick pan without the addition of any further ingredients at medium heat and kept reversed on the pan after 3 minutes in the first run, 1 minute in the second run and finally, after 15 seconds for each sides. Consequently, the complete heating duration was 8.5 min.

2.2.4. Textural analysis

Texture analysis device (TA-XT Plus, Texture Analyzer, UK) was used with a maximum load cell of 50 kg. Compression tests were performed on meatball samples and thus the texture analysis profiles (TPA) were determined. Slices with constant thicknesses (approx. 1.5 cm) were cut from meatball samples in each different group and the analyses were performed under ambient conditions (22±1°C). In these assays, 50% compression was applied to the meatball samples and the recovery rate was monitored (Crehan et al., 2000; Herrero et al., 2007; Bozkurt and Bayram, 2006). Exponent 32 software provided by the instrument manufacturer was used in the data analysis.

2.2.5. Reduction in size

Once meatball samples were weighed and portioned, their diameters were also measured as raw and cooked. The extent of size reduction (%) due to cooking was determined quantitatively using a ruler.

2.2.6. Sensory analysis

Sensory analysis of meatballs was based on appearance, hardness, juiciness, aroma and overall acceptance parameters. The panelists evaluated these parameters using a hedonic scale of 0-9, where 1-2-3 (poor), 4-5-

6 (moderate), 7-8 (good), and 9 (very good) could be rated (Barrett et al., 2010).

Seven panelists were selected among NKU Agricultural Biotechnology faculty members, undergraduate and graduate students (3 males and 4 females between the ages of 20-30). Cooked meatballs were randomly coded using numbers 1-9. Panelists evaluated a total of 9 samples in one session, including 1 control, 4 hazelnut protein bearing (5, 10, 15, and 20%) and 4 sesame protein bearing (5, 10, 15, and 20%) samples. Panelists were provided with bread and water at room temperature to clean and rinse the palate during sensory analysis.

2.2.7. Color analysis

Color analysis was conducted using a desktop spectrophotometer (Konica Minolta, CM-5, Japan) and *L*a*b** values (L, lightness; *a: redness, b: yellowness)* were determined. The total color difference (ΔE*) between control meatball and protein isolate added meatball samples was calculated by the following formula:

$$\Delta E^* = [(\Delta L^*)^2 + (\Delta a^*) + (\Delta b^*)^2]^{1/2} \quad (1)$$

2.2.8. Statistical analysis

Data on the physical, chemical and sensory properties of the samples were evaluated by one-way ANOVA tests using the JMP PRO software. The differences between the sample means of treatments were tested for significance at $p<0.05$ level with Tukey multiple comparison tests (Abdi and Williams, 2010). In all data presentations, upper index letters such as A, B, AB etc. represented significant differences between treatments at $p<0.05$ level when the upper index letters were not identical.

3. Results and discussions

3.1. Composition of protein isolates

Firstly, in order to determine the compositional attributes of hazelnut and sesame protein isolates, their corresponding moisture, ash and protein contents (%) were analyzed (Table 1). The protein content of hazelnut protein isolates (HPI) was found to be approximately 89.3%, whereas sesame protein isolates (SPI) contained approx. 81% protein. The ease of preparation and high protein content of HPI and SPI demonstrated their potential for being utilized in protein fortification of industrial food products. In addition, ash and moisture contents of the samples were in accordance with the Turkish Food Codex and EU regulation on whey protein isolate (Turkish Department of Food, Agriculture and Livestock, 2017; European Commission, 2018). Based on a modified Lowry test, protein solubility for SPI and HPI dispersions (15%) was 26% and 26.7%, respectively. The solubility of a protein isolate largely depends on the environmental pH, ionic strength, and medium temperature. The solubility of pumpkin seed proteins, for example, was reviewed to widely vary with medium pH and ionic strength (Bučko et al., 2015) while a relatively less soluble protein source could still be utilized in low moisture foods. For example, while initial moisture of various meatball formulations could be anticipated to be in the order of approx. 58-66% (Yılmaz, 2005), after cooking, meatballs had slightly lower extent of moisture, ranging mostly between 50-58% (Ulu, 2004; Serdaroğlu and Değirmencioğlu, 2004).

Table 1. % Moisture, % ash and% protein content, water holding capacity (WHC) and oil holding capacity (OHC) values for hazelnut protein isolates (HPI) or sesame protein isolates (SPI).

Samples	%Moisture	% Ash	% Protein	WHC (g/g)	OHC (g/g)
HPI	2.16±0.1	1.3±0.1	89.30±0.1	2.06±0. 1^{A}	2.59±0.1^{A}
SPI	1.65±0.1	1.43±0.1	80.95±0.1	1.96±0. 1^{B}	2.37±0.1^{B}

3.2. Water and oil holding capacities of the protein isolates

Water and oil holding capacities of HPI and SPI were presented on Table 1. The water holding capacity (WHC) of protein isolates was 2.06 and 1.96 (g/g), respectively, for HPI and SPI. Similarly, oil holding capacity (OHC) was 2.59 and 2.37 (g/g) for the same samples. According to these findings, OHC values of the isolates were higher than their WHC counterparts, which could improve the duration of aroma in food products such as baked goods including cakes and biscuits (Khalil et al., 2001). Relatively high OHC values could also prove instrumental upon grilling of meat products. In addition, these protein products could extend the shelf life and improve the flavor of the products by reducing water and fat loss in meat products (Guerrero et al., 2002). Consequently, an attempt was made here for their usage in meatballs. Compared to the previous works of our group, WHC and OHC values of the current samples were higher than that of black cumin protein isolates (Coşkun et al., 2019). These differences could be influenced by the processing or extraction methods, final protein composition and concentration in the samples as well as the nature of the impurities present. While the current OHC value for SPI was higher than that obtained by Khalid et al. (2003), no hexane treatment was administered in this study, which could preserve the native structure of proteins and affect the oil and/or water holding characteristics.

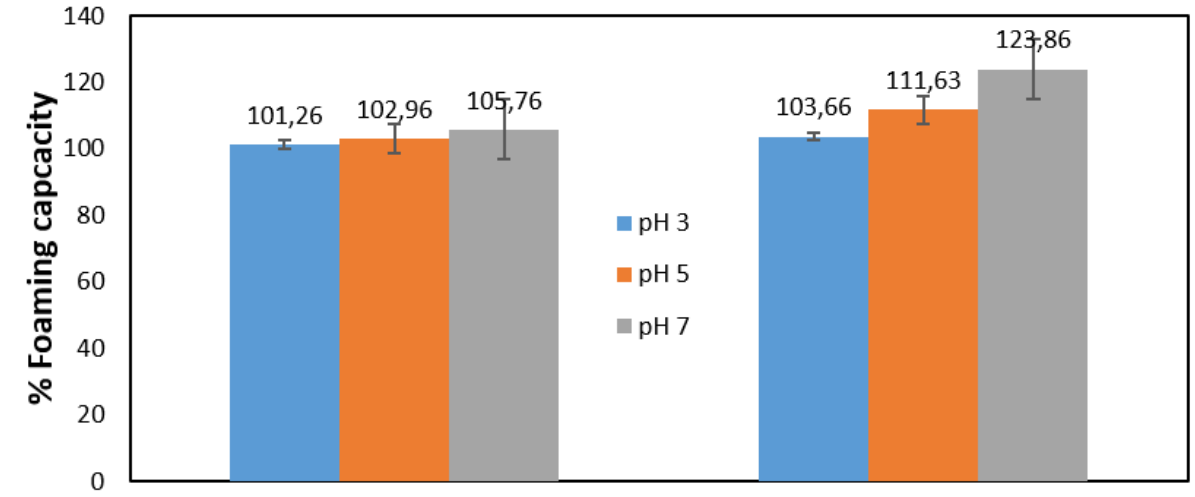
3.3. Foam formation and stabilization capacities of the protein isolates

Foams are defined as 2-phase dispersions consisting of air cells separated by a thin layer of liquid. In the stabilization of foams, surface active molecules including proteins are needed to reduce the surface tension at the air/water surface (Makri et al., 2005). Foam formation and stability are strongly dependent on foam preparation method, composition and concentration of proteins, medium pH and ionic strength, and hydrophobic interactions (Massoura et al., 1998). While globular proteins demonstrate low foaming ability due to limited surface denaturation, flexible protein molecules tend to perform highly in foam formation (Kaur and Singh, 2007).

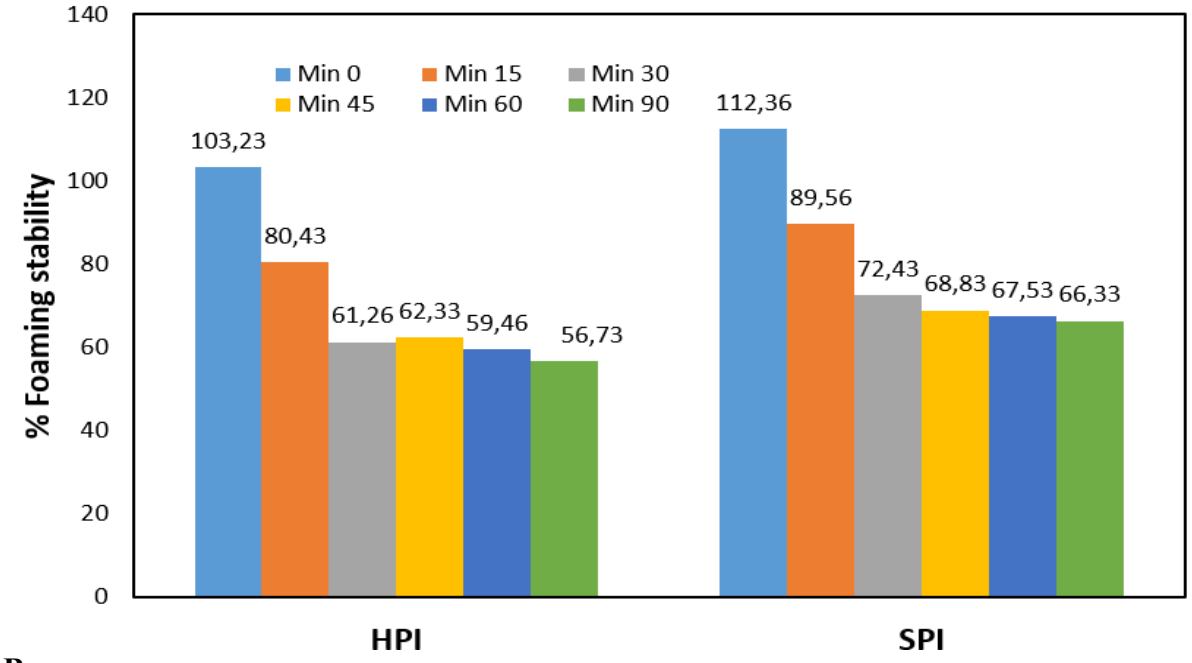
Due to the average pH value range of food products (primarily pH 3 to 7), the capacity of protein isolate dispersions (5%, 50 ml) to generate foams was considered in this pH range (Figure 2A). Generally, SPI was found to be a better foaming agent compared to HPI ($p< 0.05$). In the current pH range, the foaming capacity of both hazelnut and sesame proteins increased with pH as the overall negative charge density increased ($p<0.05$). While charge density increases, hydrophobic interactions in protein molecules may be anticipated to weaken and consequently, proteins gain a relatively more flexible structure that could facilitate foam formation (Guerrero et al., 2002; Aluko, 2004). SPI dispersions at pH 7 generated the highest foaming capacity. In most cases, however, there were no statistically significant differences between the stability values of SPI or HPI stabilized foams ($p>0.05$). In both cases, foam volume decreased from approx. A volume of 110 ml to approx. 55-65 ml range during a 90-minute storage process (Figure 2B).

3.4. Emulsion formation and stabilization capacities of the protein isolates

Surface hydrophobicity and protein concentration are the main characteristics that determine the properties of protein stabilized emulsions. Emulsification activity index (EAI) reflects the ability of a certain protein to adsorb to the oil/water interface during emulsion formation. The emulsion stability index (ESI) is a measure of the ability of a certain protein to provide stable emulsions for a certain period of time (Subagio, 2006). The emulsification activity and emulsion stability of the current samples were shown on Table 2. EAI values were determined as approximately $175 m^2.g^{-1}$ for sesame protein isolates. EAI values of sesame protein stabilized emulsions have decreased slightly over time. EAI values for hazelnut samples were found to be significantly higher (approximately 294 $m^2. g^{-1}$). The decrease in ESI and EAI values has been limited in all cases. While further optimization could enhance the foaming and emulsification attributes, the current findings were coherent with the findings of Cano-Medina et al. (2011) on sesame proteins.



A



B

Figure 2. (A) Foam formation capacities of HPI or SPI (5%) as a function of pH. **(B)** Foam stability as a function of time (0-90 min, pH 7).

Table 2. (A) Emulsion formation activities and stability index values for the HPI and SPI dispersions (5%, 50 ml) as a function of time (0-120 min).

	HPI		SPI	
t (min)	**EAI ($m^2.g^{-1}$)**	**ESI (%)**	**EAI ($m^2.g^{-1}$)**	**ESI (%)**
0	294.46 ± 0.1^{A}	49.16 ± 0.1^{A}	175.56 ± 0.1^{A}	49.33 ± 0.1^{A}
30	294.16 ± 0.1^{B}	49.03 ± 0.1^{A}	175.53 ± 0.1^{A}	49.26 ± 0.1^{A}
45	293.4 ± 0.1^{C}	48.76 ± 0.1^{B}	175.33 ± 0.1^{A}	48.83 ± 0.1^{B}
60	293.42 ± 0.1^{C}	48.53 ± 0.1^{C}	174.80 ± 0.1^{B}	48.66 ± 0.1^{C}
120	293.23 ± 0.1^{C}	48.16 ± 0.1^{D}	174.53 ± 0.1^{C}	48.53 ± 0.1^{C}

To summarize the findings, it must be noted that protein concentration in HPI samples were higher than their sesame counterparts. While solubility (%) values were comparable, WHC and OHC values of HPI samples were slightly higher. Foaming attributes of SPI samples were more significant, whereas emulsion formation capabilities of HPI were higher. The differences in the performances of HPI and SPI could be attributed to their structural differences and molecular sizes. For hazelnut proteins, the majority of 1D and 2D electrophoretic bands were found to lie between approx. 18 to 25 kDa, while their corresponding isoelectric points primarily were between pH 5-8.5 (Aydemir et al., 2014). Mostly comparable molecular weight results were obtained by Saricaoglu et al. (2018). The major bands for sesame proteins were identified around 35 and 75 kDa (Singharaj and Onsaard, 2015). Native-PAGE analysis indicated much larger aggregates in sesame proteins (11S), while the corresponding isolates were predominantly alpha-helical (Achouri et al., 2012). The authors commented that while the WHC, OHC and solubility attributes of sesame proteins were relatively less pronounced, emulsifying and foaming characteristics were relatively higher than soy protein isolate (Achouri et al., 2012). Under various extraction conditions, isoelectric points of the sesame proteins were mostly between pH 4-5 (Achouri et al., 2012). The molecular sizes and other structural attributes might lead to the differences in the performances of the current samples. In addition, composition of impurities in the isolates could also affect the results. Since the ash and moisture contents of the current samples were similar, fiber composition of the protein isolates may also be considered. Hazelnuts were found to be characterized with a total fiber content of approx. 12.9%, most (10.67%) of which was insoluble fiber (Alasalvar et al., 2003). In most studies, the crude fiber content of sesame seeds was expressed as <5-6% (Kryuchkova et al., 2021). Consequently, molecular size characteristics and fiber content of the two samples can be anticipated to be significantly different.

While physico-chemical properties of sesame proteins and to a lesser extent that of hazelnut proteins, were known, the current findings on current isolates were comparable to the previous literature. Based on these data, in the next stages of the study, sustainable plant protein fortification in meatball manufacture was investigated.

3.5. Analysis of meatballs fortified with protein isolates

In this section, the influence of HPI or SPI fortification on raw and/or cooked meatball characteristics are being summarized.

3.5.1. Reduction in size

Since the characteristic sizes of foods change during processing, the diameter values of the meatball samples were determined before and after preparation and the extent of size reduction (%) was calculated (Figure 1, Table 3). Similar extents of size reduction were observed at different protein inclusion rates for SPI, while the size changes were non-identical at various HPI concentrations. The highest extent of size reduction in meatball samples was generated by 10% hazelnut protein inclusion, whereas the least extent of size reduction took place for 5% hazelnut protein bearing meatball samples. There was no clear relationship between samples with different concentrations of protein isolates and in most cases, reduction in size was approx. in the order of 20% and the extent of reduction was mostly comparable to the control sample (i.e., 17.55%). However, in all cases including the controls, diameter reduction was more significant compared to low-fat meatballs fortified with blackeye bean flour, chickpea flour, lentil flour or rusk, where diameter reduction was <11% (Serdaroğlu et al., 2005).

3.5.2. Textural analysis

Textural profile analysis (TPA) was performed in order to detect changes in the textural attributes of the meatballs due to fortification with HPI or SPI (Table 4). Primarily, firmness and toughness parameters were examined. The firmness values for all samples were found to range between 2135 and 3375 g. The firmness value was determined to be the highest in 15% HPI bearing samples, while the lowest value was determined in the 10% HPI bearing counterpart (Table 4). In

general, the firmness values of meatball samples with varying concentrations of SPI or HPI were higher than the control and there were statistically significant differences between the samples ($p<0.05$). Previously, bacterial cellulose addition was shown to lower firmness of Chinese-style cooked meatballs as investigated by sensory analysis (Lin and Lin, 2004). In the current studies, the addition of protein isolates did not cause any statistically significant differences in toughness values ($p>0.05$).

Table 3. Cooking related size reduction (%) in meatball diameter as a function of HPI or SPI fortification (5-20%).

Sample	% Size Reduction
Control	17.55 ± 1.3^{B}
%5 SPI	20 ± 0.1^{AB}
%10 SPI	21.9 ± 0.6^{A}
%15 SPI	20 ± 0.01^{AB}
%20 SPI	21.9 ± 0.6^{A}
%5 HPI	23.15 ± 0.6^{A}
%10 HPI	20.65 ± 0.6^{AB}
%15 HPI	21.9 ± 0.6^{A}
%20 HPI	20.65 ± 0.6^{AB}

Table 4. Textural parameters (firmness and toughness) of cooked meatballs as a function of HPI or SPI fortification (5-20%).

	Firmness	Toughness
Control	2964 ± 130^{AB}	27874 ± 2831^{A}
%5 SPI	2416 ± 146^{BC}	31437 ± 2986^{A}
%10 SPI	2136 ± 174^{C}	24103 ± 3507^{A}
%15 SPI	3375 ± 36^{A}	30709 ± 312^{A}
%20 SPI	2517 ± 62^{BC}	23082 ± 653^{A}
%5 HPI	2388 ± 128^{BC}	24617 ± 2690^{A}
%10 HPI	2550 ± 49^{BC}	27127 ± 513^{A}
%15 HPI	2714 ± 70^{BC}	23083 ± 882^{A}
%20 HPI	2875 ± 39^{AB}	26564 ± 2071^{A}

3.5.3. Color analysis

The color attributes (L*, a*, b*) of meatball products fortified with HPI or SPI were given on Table 5. L* values decreased and the sample colors become visibly darker upon the addition of SPI. The L* value slightly increased with the addition of 10% HPI, and beyond that L* value decreased again. Most of the changes related to L* were not statistically significant. ΔE values express the overall color change, where color differences corresponding to $\Delta E^*>3$ are clearly visible to the naked eye (Jarpa-Parra et al.,

2017). In a number of cases, ΔE values were found to be >3 (Table 5). In terms of total color change, there was a significant difference between SPI and HPI treatments ($p<0.05$). Therefore, the influence of each protein isolate should be evaluated separately for the products.

Table 5. Color parameters for cooked meatballs as a function of HPI or SPI fortification (5-20%).

Sample	*L**	*a**	*b**	*ΔE*
Control	38.77 ± 0.2^{BC}	4.07 ± 0.2^{AB}	4 ± 0.2^{B}	N/A
%5 SPI	43.19 ± 0.2^{A}	3.87 ± 0.2^{ABC}	7.2 ± 0.1^{A}	5.46
%10 SPI	41.69 ± 0.3^{ABC}	3.28 ± 0.3^{BCD}	6.23 ± 0.4^{AB}	3.76
%15 SPI	38.77 ± 0.1^{BC}	2.75 ± 0.1^{D}	4.34 ± 1.3^{B}	1.36
%20 SPI	40.87 ± 0.2^{ABC}	4.21 ± 0.2^{A}	5.84 ± 0.5^{AB}	2.80
%5 HPI	38.15 ± 0.1^{C}	3.42 ± 0.1^{ABCD}	3.6 ± 0.1^{B}	0.99
%10 HPI	42.17 ± 0.2^{AB}	3.13 ± 0.2^{CD}	6.44 ± 0.3^{AB}	4.29
%15 HPI	39.07 ± 0.1^{BC}	2.78 ± 0.1^{D}	4.16 ± 0.1^{B}	1.34
%20 HPI	39.37 ± 0.1^{ABC}	3.52 ± 0.1^{ABCD}	4.35 ± 0.1^{AB}	0.89

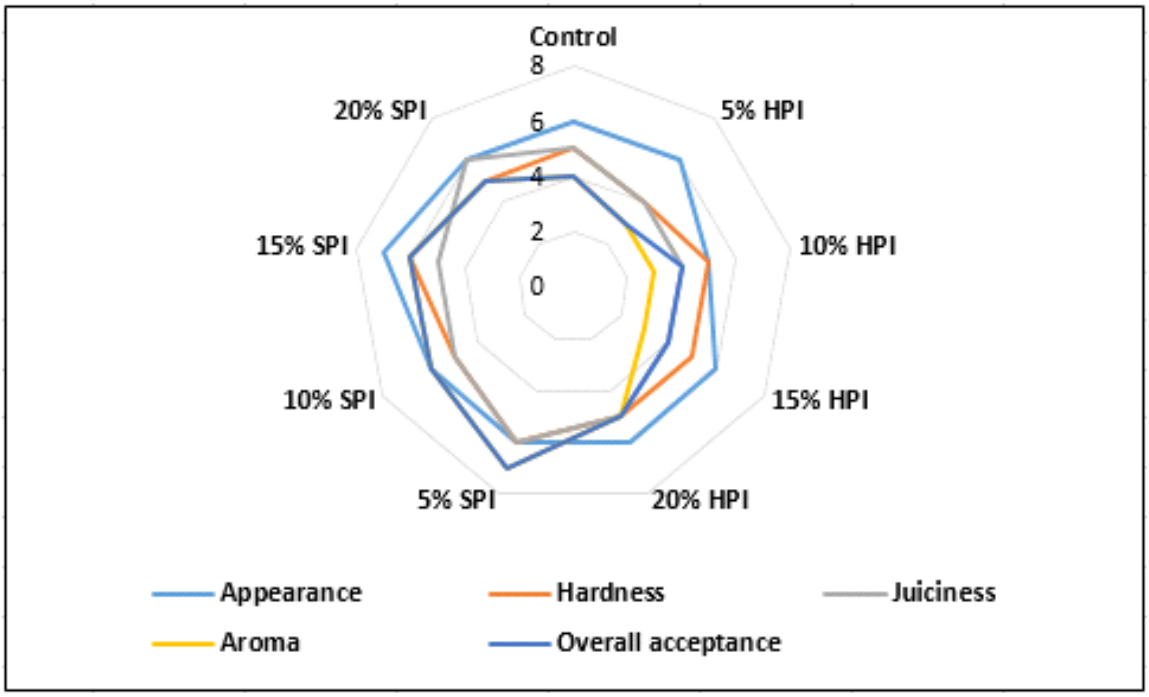


Figure 3. Sensory analysis of the meatball products after protein fortification with HPI or SPI (5-20%).

3.5.4. Sensory analysis

Meatballs fortified with different concentrations of HPI and SPI were analyzed by sensory methods and the results were presented on Figure 3. Sensory analysis was based on a number of sensory characteristics including appearance, hardness, juiciness, aroma and overall acceptance. The changes in appearance, hardness, juiciness, aroma and general acceptance values were statistically significant to the panelists upon the addition of different concentrations of HPI or SPI (5-20%) ($p<0.05$).

The panelists rated the samples that contained 5% SPI with the highest scores. Panelists have indicated that they would also rate meatballs containing 10% and 15% SPI as acceptable, whereas 5% or 15% HPI

samples and control samples were preferred at a lower rate. The acceptability of 5% SPI fortified meatballs were attributed primarily to their aroma characteristics. While bran addition to meatballs up to 10% did not alter sensory analysis results significantly, the authors emphasized the influence of particle size on sensory attributes (Huang et al., 2005). Consequently, the current results could be further enhanced based on the optimization of fortification procedures.

4. Conclusions

Cold press oil production has become increasingly popular over the recent years, which in turn lead to the generation of high quality (i.e., low oil content, low peroxide value etc.) and high-protein content press cakes. Consequently, the valorization of seed cakes has gained importance. In this study, protein isolates were generated from sesame and hazelnut press cakes and utilized in meatball manufacture. While the differences between two protein isolates were attributed to differences in molecular sizes and potential differences in fiber content, a certain protein fortification level (mostly 5%) was acceptable. Proteins generate molecular interactions amongst each other, hold significant amounts of water and fat, consequently protein fortification of meat products could easily generate an undesirable texture. In the current study, however, while protein fortification altered the color of the meatballs and increased firmness, toughness or size reduction attributes were mostly unaffected. Plant protein isolates can be utilized in either partial replacement of meat protein or in fortification of protein content and generate more sustainable products.

5. References

Abdi, H., Williams, L.J. (2010). Contrast analysis. *Encyclopedia of Research Design*, 1, 243-251.

Achouri, A., Nail, V., Boye, J.I. (2012). Sesame protein isolate: Fractionation, secondary structure and functional properties. *Food Research International*, 46(1), 360-369.

Alasalvar, C., Shahidi, F., Liyanapathirana, C.M., Ohshima, T. (2003). Turkish tombul hazelnut (*Corylus avellana* L.). 1. Compositional characteristics. *Journal of Agricultural and Food Chemistry*, 51(13), 3790-96.

Aluko, R. E. (2004). The extraction and purification of proteins: an introduction, *In* Proteins in Food Processing, pp. 323-346, Ed. R.Y. Yada, Woodhead Publishing Ltd., Cambridge, England.

Aydemir, L.Y., Gökbulut, A.A., Baran, Y., Yemenicioğlu, A. (2014). Bioactive, functional and edible film-forming properties of isolated hazelnut (*Corylus avellana* L.) meal proteins. *Food Hydrocolloids*, 36, 130-142.

Barrett, D.M., Beaulieu, J.C., Shewfelt, R. (2010). Color, flavor, texture, and nutritional quality of fresh-cut fruits and vegetables: desirable levels, instrumental and sensory measurement, and the effects of processing. *Critical Reviews in Food Science and Nutrition*, 50(5), 369-389.

Bozkurt, H., Bayram, M. (2006). Colour and textural attributes of sucuk during ripening. *Meat Science*, 73, 344-350.

Bučko, S., Katona, J., Popović, L., Vaštag, Ž., Petrović, L., Vučinić–Vasić, M. (2015). Investigation on solubility, interfacial and emulsifying properties of pumpkin (*Cucurbita pepo*) seed protein isolate. *LWT- Food Science and Technology*, 64(2), 609-615.

Çağlar, A.F., Çakır, B., Gülseren, İ. (2021). LC-Q-TOF/MS based identification and *in silico* verification of ACE-inhibitory peptides in Giresun (Turkey) hazelnut cakes. *European Food Research and Technology*, 247(5), 1189-1198.

Cano-Medina, A., Jiménez-Islas, H., Dendooven, L., Herrera, R.P., González-Alatorre, G., Escamilla-Silva, E. M. (2011). Emulsifying and foaming capacity and emulsion and foam stability of sesame protein concentrates. *Food Research International*, 44(3), 684-692.

Chabanon, G., Chevalot, I., Framboisier, X., Chenu, S., Marc, I. (2007). Hydrolysis of rapeseed protein isolates: kinetics, characterization and functional properties

of hydrolysates. *Process Biochemistry*, 42, 1419-1428.

Coşkun, Ö., Çakır, B., Vahapoğlu, B., Gülseren, İ. (2019). Influence of extraction conditions on structural and functional characteristics of black cumin protein concentrates and ACE-inhibition in their hydrolyzates. *Journal of Food Measurement and Characterization*, 13(3), 2328-2338.

Coşkun, Ö., Pehlivanoğlu, H., Gülseren, İ. (2020). Pilot scale assessment for seed protein enrichment of gluten-free breads at varying water content levels and after protein modification treatments. *Journal of Food Processing and Preservation*, 44(7), e14512.

Crehan, C.M., Hughes, E., Troy, D.J., Buckley, D.J. (2000). Effects of fat level and maltodextrin on the functional properties of frankfurters formulated with 5, 12 and 30 % fat. *Meat Science*, 55, 463-469.

Da Silva, P.C., Toledo, T., Brião, V., Bertolin, T.E., Costa, J.A.V. (2021). Development of extruded snacks enriched by bioactive peptides from microalga *Spirulina* sp. LEB 18. *Food Bioscience*, 42, 101031.

Day, L. (2013). Proteins from land plants – potential resources for human nutrition and food security. *Trends in Food Science and Technology*, 32, 25-42.

European Commission (2018). Commission Implementing Regulation (EU) 2018/1632 of 30 October 2018 authorizing of the placing on the market of bovine milk basic whey protein isolate as a novel food under Regulation (EU) 2015/2283 of the European Parliament and of the Council and amending Commission Implementing Regulation (EU) 2017/2470 https://eur-lex.europa.eu/legal-content/EN/TXT/?uri=CELEX%3A32018R1632 (accessed, 06.06.2021).

Fallico, B., Arena, E., Zappala, M. 2003. Roasting of hazelnuts. Role of oil in colour development and hydroxymethylfurfural formation. *Food Chemistry*, 81, 569–573.

Guerra, I. C. D., Felex, S.S.S., Meireles, B. R.L.M., Dalmas, P. S., Moreira, R. T.,…, Madruga, M. S. (2011). Evaluation of goat mortadella prepared with different levels of fat and goat meat from discarded animals. *Small Ruminant Research*, 98, 59–63.

Guerrero, L.C., Perez-Flores, V., Betancur-Ancona, D., Davilla-Ortiz, G. (2002). Functional properties of flours and protein isolates from *Phaseolus lunatus* and *Canavalia ensiformis* seeds. *Journal of Agricultural Food Chemistry*, 50, 584-591.

Herrero, A.M., Ordónez, J.A., de Avila, R., Herranz, B., de la Hoz, L., Cambero, M.I. (2007). Breaking strength of dry fermented sausages and their correlation with texture profile analysis (TPA) and physico-chemical characteristics. *Meat Science,* 77, 331-338.

Huang, S.C., Shiau, C.Y., Liu, T.E., Chu, C.L., Hwang, D.F. (2005). Effects of rice bran on sensory and physico-chemical properties of emulsified pork meatballs. *Meat Science*, 70(4), 613-619.

Jarpa-Parra, M., Wong, L., Wismer, W., Temelli, F., Han, J., Huang, W., … Chen L. (2017). Quality characteristics of angel food cake and muffin using lentil protein as egg/milk replacer. *International Journal of Food Science and Technology*, 52(7), 1604–1613.

Kaur, M., Singh, N. (2007). Characterization of protein isolates from different Indian chickpea (*Cicer arietinum* L.) cultivars. *Food Chemistry*, 102(1), 366-374.

Khalid, E.K., Babiker, E.E., Tinay, A.E. (2003). Solubility and functional properties of sesame seed proteins as influenced by pH and/or salt concentration. *Food Chemistry*, 82(3), 361-366.

Khalil, M.M. (2001). Biochemical and technological studies on the production of the isolated guar protein. *Nahrung*, 45, 21-24.

Kryuchkova, V.V., Gorlov, I.F., Slozhenkina, M.I., Lomonova, N.V., Belik, S.N. (2021). Functional curd product of increased biological value. *In* IOP Conference Series: Earth and

Environmental Science (Vol. 677, No. 3, p. 032073). IOP Publishing.

Lin, K.W., Lin, H.Y. (2004). Quality characteristics of Chinese-style meatball containing bacterial cellulose (Nata). *Journal of Food Science*, 69(3), SNQ107-SNQ111.

Makri, E., Papalamprou, E., Doxastakis, G. (2005). Study of functional properties of seed storage proteins from indigenous European legume crops (lupine, pea, broad bean) in admixture with polysaccharides. *Food Hydrocolloids*, 19, 583-594.

Massoura, E, Vereijken, J.M., Kolster, P., Derksen, J.T.P. (1998). Proteins from *Crambe abyssinica* oilseed II: Biochemical and functional properties. *Journal of American Oil Chemists` Society*, 75, 329-335.

Moure, A., Rua, M., Sineiro, J., Dominguez, H. (2001). Extraction and functionality of membrane-concentrated protein from defatted *Rosa rubiginosa* seeds. *Food Chemistry,* 74, 327-339.

Ogunwolu, S.O., Henshaw, F.O., Mock, H. P., Santros, A., Awonorin, S.O. (2009). Functional properties of protein concentrates and isolates produced from cashew (*Anacardium occidentale* L.) nut. *Food Chemistry*, 115, 852–858.

Parker, T.D., Adams, D.A., Zhou, K., Harris, M., Yu, L. (2003). Fatty acid composition and oxidative stability of cold-pressed edible seed oils. *Journal of Food Science*, 68, 1240-1243.

Prasad, N., Sanjay, K.R., Prasad, D.S., Vijay, N., Kothari, R., Nanjunda Swamy, S. (2012). A review on nutritional and nutraceutical properties of sesame. *Nutrition and Food Science*, 2(2), 1000127.

Saget, S., Costa, M., Santos, C.S., Vasconcelos, M.W., Gibbons, J., Styles, D., Williams, M. (2021). Substitution of beef with pea protein reduces the environmental footprint of meat balls whilst supporting health and climate stabilisation goals. *Journal of Cleaner Production*, 297, 126447.

Sanchez-Vioque, R., Clemente, A., Vioque, J., Bautista, J., Millan, F. (1999). Protein isolates from chickpea (*Cicer arietinum* L.): chemical composition, functional properties and protein characterization. *Food Chemistry*, 64, 237-243.

Saricaoglu, F.T., Gul, O., Besir, A., Atalar, I. (2018). Effect of high pressure homogenization (HPH) on functional and rheological properties of hazelnut meal proteins obtained from hazelnut oil industry by-products. *Journal of Food Engineering*, 233, 98-108.

Seena, S., Sridhar, K.R. (2005). Physiochemical, functional and cooking properties of Canavalia. *Journal of Food Chemistry,* 32, 406–412.

Serdaroğlu, M., Değirmencioğlu, Ö. (2004). Effects of fat level (5%, 10%, 20%) and corn flour (0%, 2%, 4%) on some properties of Turkish type meatballs (koefte). *Meat Science*, 68(2), 291-296.

Serdaroğlu, M., Yıldız-Turp, G., Abrodímov, K. (2005). Quality of low-fat meatballs containing legume flours as extenders. *Meat Science*, 70(1), 99-105.

Singharaj, S., Onsaard, E. (2015). Production and characteristic of sesame proteins. *Journal of Food Science and Agricultural Technology*, 1, 188-192.

Subagio, A. (2006). Characterization of hyacinth bean (*Lablab purpureus* (L.) sweet) seeds from Indonesia and their protein isolate. *Food Chemistry*, 95(1), 65-70.

Turkish Ministry of Food, Agriculture and Livestock (2017). Regulation No. 30188 amending the Turkish Food Codex Regulation on Food Additives. No: 30188 (accessed, 06.06.2021)

Ulu, H. (2004). Effect of wheat flour, whey protein concentrate and soya protein isolate on oxidative processes and textural properties of cooked meatballs. *Food Chemistry*, 87(4), 523-529.

Yağcı, S., Göğüş, F. (2008). Response surface methodology for evaluation of physical and functional properties of extruded snack foods developed from

food-by-products. *Journal of Food Engineering*, 86(1), 122-132.

Yılmaz, E. (2015). Consumer food safety knowledge, practices and differences in behaviors in Thrace region of Turkey. *Journal of Agricultural Science*, 21(2), 279-287.

Yılmaz, I. (2005). Physicochemical and sensory characteristics of low fat meatballs with added wheat bran. *Journal of Food Engineering*, 69(3), 369-373.

Zhang, W., Liu, C., Zhao, J., Ma, T., He, Z., Huang, M., Wang, Y. (2021). Modification of structure and functionalities of ginkgo seed proteins by pH-shifting treatment. *Food Chemistry*, 358, 129862.

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