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Fractionation and characterization of heat-stable basil seed protein isolates and their utilization in high protein gluten-free bread

Mohammad Rashid[1] | **İbrahim Gülseren**[1,2,3]

[1]Department of Food Engineering, İstanbul S. Zaim University (İZÜ), Sabri Ülker R&D Center Bldg., Istanbul, Turkey

[2]İZÜ Food and Agricultural Research Center (GTUAM), Halkalı Campus, Sabri Ülker R&D Center Bldg., Istanbul, Turkey

[3]Functional Ingredient Technologies (FonkBilTek) R&D and Consulting Services Ltd. Co., Zaim Technopark, İZÜ Halkalı Campus, Istanbul, Turkey

Correspondence
İbrahim Gülseren, Department of Food Engineering, İstanbul S. Zaim University (İZÜ), Sabri Ülker R&D Center Bldg., Halkalı – Küçükçekmece, Istanbul, Turkey.
Email: ibrahim.gulseren@izu.edu.tr

Abstract

Basil (*Ocimum basilicum* L.) is an annual plant known for its essential oil and phenolic content. The aim of this study was to isolate and characterize proteins from basil seeds and evaluate their potential in food fortification. The mucilage was removed from the seed by aqueous extraction and thermal hydration, whereas oil was removed via cold pressing. Basil seed protein isolate (BSPI) was obtained by an alkali extraction-isoelectric precipitation method and contained approximately 95% protein. Once BSPI was fractionated, the samples were characterized by albumin (13.5%), globulin (16.7%), glutelin (39.5%), and prolamin (30.2%) fractions. The denaturation temperature of BSPI and its fractions ranged between 93 and 142°C. Through the analysis of Fourier transform infrared spectra, β-sheet and β-turn elements were found to be dominant secondary structures accounting for 89.4% in BSPI, which in turn lead to enhanced thermal stability. Since aqueous dispersibility and water holding capacity (WHC) values were acceptable, BSPI was further utilized in the manufacture of gluten-free bread. The textural parameters of bread supplemented with 2%, or 10% basil seed protein were mostly comparable to the controls rendering basil protein fortification possible in a staple food, especially for countries where basil is produced in high quantities and is reasonably affordable.

KEYWORDS
basil seeds, DSC, FT-IR spectroscopy, gluten-free bread, high protein foods, Osborne fractionation, textural analysis

INTRODUCTION

Due to the increasing global population, the cost of animal proteins is predicted to increase all over the world (FAO, 2013). Based on this prediction, various researchers are seeking new alternatives that can meet the protein demand. In that regard, different plant protein sources (i.e., seeds, grains, and leaves) have been heavily investigated in the current literature (Adenekan et al., 2018; Eltayeb et al., 2011; Pojić et al., 2018; Sun et al., 2012). Plant proteins are sustainable sources suitable for human consumption. The conversion rate of plant proteins to animal protein in livestock was predicted to be approximately 15% (Yimer et al., 2019). Although plant proteins are more abundant and cheaper than animal proteins, their industrial use in food systems remains relatively limited (Hertzler et al., 2020). The functional properties of plant proteins (Owusu-Apenten, 2004) might have potentially lead to this outcome. However, in addition to the replacement of animal proteins, for example, in dairy-free or meat-free product categories, recent studies on gluten-free products highlighted the importance of economically viable, sustainable, and technologically functional plant protein products (Day, 2013).

The functional properties of food proteins are the physical and chemical properties that affect the proteins during the production, storage, and consumption

of food (Owusu-Apenten, 2004). For example, wheat gluten is an important protein group in dough due to its contributions to flexibility and plasticity of dough. Breadmaking qualities of wheat flour depends on both the amount and quality of gluten proteins (Arendt et al., 2008). Permanent lifelong avoidance of gluten from the diet is the only effective treatment for celiac disease. However, the removal of gluten from bread formulations often results in a dough lacking optimal viscoelastic properties in the prebaking step and can result in baked bread with crumbly texture, poor color, and other quality defects. Most gluten-free products currently on the market are of poor quality, taste, and significantly limiting protein content (Arendt et al., 2002). Gluten-free bread requires polymeric substances that mimic the viscoelastic properties of gluten in bread dough (Toufeili et al., 1994).

Basil (*Ocimum basilicum* L.) is an annual, 20–60 cm tall, green herb belonging to the Lamiaceae family. China and India are among its major producers, while basil is mostly grown in tropical, subtropical, and temperate regions (Shahrajabian et al., 2020). The current magnitude of global basil seed economy is around 90 million USD. The seed is elliptical with an average size of approximately 2.5 mm in length, 1.4 mm in width, and 1.1 mm in thickness and composed of approximately 7%–10% moisture, 17%–29% protein, 15%–33% oil, 5%–7% ash, 40%–50% carbohydrates, and 7% fiber (Khaliq et al., 2017; Munir et al., 2017; Nazir & Wani, 2021). Basil seeds and components have been used as thickeners, fat replacers, stabilizers, emulsifiers, foaming, and gelling agents in the food industry (Choi et al., 2020; Gajendiran et al., 2016; Nazir et al., 2017). In some parts of Asia, basil seeds are utilized in the preparation of desserts and beverages. The chemical constituents of the basil seed provide it with antimicrobial, anticancer (Gajendiran et al., 2016), antioxidant, anthelmintic, anti-inflammatory (Decker et al., 2010), antihyperglycemic (Chaudhary et al., 2016), and antiseptic activities. In addition, basil seeds have been used in traditional medicine for the treatment of cough, headache, diarrhea, stomachache, constipation, and skin infections (Shahrajabian et al., 2020). However, the literature on the characterization and potential uses of basil seed proteins is relatively scarce, while basil seed gums have been keenly investigated (Avlani et al., 2019; Hussain et al., 2019). A complementary approach can be taken in order to simultaneously valorize both gums and proteins from oil processing by products.

In bakery applications, bread quality primarily depends on both the amount and quality of gluten proteins, which constitute 80%–85% of total wheat proteins (Arendt et al., 2008). Their major functionalities include providing dough viscoelasticity, and superior gas holding characteristics (Arendt et al., 2008). At this time, permanent avoidance of gluten is the only effective treatment for celiac disease. However, removal of gluten from bread often results in a liquid dough at the prebaking stage and can result in baked bread with crumbly texture, poor color, and other quality defects. Most gluten-free products suffer from low quality, tastelessness, limited protein content, and potentially high glycemic index (Arendt et al., 2002). These problems lead the researchers to the pursuit for gluten alternatives that can reasonably mimic the viscoelastic properties of gluten network in bread dough (Toufeili et al., 1994). In the earlier studies of our group, plant protein fortification of gluten-free bread was characterized with increased hardness and water holding capacity, which could be counterbalanced by water addition to the dough (Coşkun et al., 2020).

The aim of this study was to obtain protein isolates from cold press basil seed cakes, and valorize them in high protein, gluten-free bread manufacture. Based on the widespread availability and relatively low cost of basil seeds, affordable and protein-rich bread formulations could become a staple food in various countries. Consequently, protein content of the isolates was determined and their physico-chemical characteristics were investigated. In addition, basil seed proteins were fractionated and the structural characteristics of Osborne fractions were investigated. Finally, textural properties of the bread samples were analyzed and the relevance of protein structure to gluten-free bread texture was discussed.

MATERIALS AND METHODOLOGIES

Materials

Basil (*Ocimum basilicum* L.) seeds were acquired from a local company (İntfa Agricultural Products, Konya, Turkey) and stored frozen in airtight containers (−20°C) until further use. Using a cold press device (Karaerler nf100, Karaerler Ltd., Ankara, Turkey), basil oil was extracted from the seeds. The maximum temperature in this process was <40°C. All chemicals used in this study were reagent-grade (Sigma-Aldrich, Schnelldorf, Germany).

Methodologies

Removal of basil seed mucilage

Basil seed mucilage was extracted from seeds by aqueous extraction and thermal hydration methods as described by Hussain et al. (2019) and Avlani et al. (2019). First, the seeds were hydrated in distilled water at ambient temperature for 1 h at a mixing ratio of 1:20 (g seed:mL water). Immediately afterwards, the mixture was kept stirred using a mechanical stirrer at 1500 × rpm for 2 h (30°C) and swollen seeds were collected. The mixture was centrifuged at 12,000*g* using a

CR22 N high-speed centrifuge (Hitachi Koki Co., Ltd., Tokyo, Japan) for 20 min to separate the seeds from excess water (25°C). Finally, the swollen seeds were dried at 45°C for 48 h and the mucilage was separated from the seeds manually using shear forces. For this purpose, the samples (50 g) were manually rubbed against a perforated metal filter for 5 min, which has enabled the separation.

Manufacture of protein isolates

Basil seeds were cold pressed at ambient temperature at an extruder rate of 18 Hz. Thus, obtained press cakes were utilized in the manufacture of basil seed protein isolates (BSPI) using an alkali extraction-isoelectric precipitation (AE-IP) method (Coşkun et al., 2019). Press cakes were mixed with water at a ratio of 1:15 (g cake:mL solvent) and medium pH was set to 9.5 to ensure dissolution. The mixture was kept stirred at 500 rpm for 1 h.

The mixture was then centrifuged (12,000*g*) for 20 min at 4°C. After centrifugation, the supernatant was collected and medium pH was adjusted to pH 4.5 to promote isoelectric precipitation. Immediately afterwards, the supernatant was re-centrifuged and thus obtained precipitates were lyophilized using a TRS 2/2 V freeze-dryer (Teknosem A.Ş., İstanbul, Turkey). Lyophilized protein isolates were stored at −20°C until further use.

Protein determination

The protein content in the samples was determined using VELP NDA 701 Dumas nitrogen analyzer (Velp, Shanghai, China) according to the AOAC method 992.15. Dumas analysis was carried out by an accredited laboratory (LOTUSLAB, Accreditation No: AB-1130-T). They have used a standardized conversion factor (N) of 6.25 in their assays. When an alternative determination method was necessary for diluted samples, protein content (%) was determined using an assay kit based on the Lowry method (TP0300, Sigma Aldrich Corp.). When necessary, bovine serum albumin (BSA) solutions were used as a standard for the Lowry assays.

Osborne fractionation

BSPI fractions were prepared according to the method of Osborne et al. (1914) with appropriate modifications (Deb et al., 2022). First of all, BSPI was mixed with distilled water at a ratio of 1:20 (g to mL) for 2 h. The suspension was then centrifuged at 12,000*g* for 20 min. The supernatant was labeled as the solution containing the albumin fraction. The precipitate (X1) was lyophilized, and preserved for further fractionation.

X1 was mixed with 5% NaCl solution at a ratio of 1:20 (g to mL) and kept stirred for 1 h. Following this process, the suspension was centrifuged at 12,000*g* for 20 min. The supernatant was labeled as the solution containing the globulin fraction. The precipitate (X2) was washed thrice with distilled water to facilitate desalination prior to lyophilization.

Immediately afterwards, lyophilized X2 was mixed with 0.1 M NaOH solution at a ratio of 1:20 (g to mL) for 1 h and based on similar processes as before, glutelin bearing supernatant and a final precipitate were recovered (X3). Once again, washing with pure water was carried out in order to remove residues of NaOH. Finally based on similar procedures, freeze-dried X3 was mixed with 70% ethanol for 4 h and the supernatant bearing the prolamin fraction was collected.

Investigation of physico-chemical properties of BSPI and its fractions

The thermal and structural analysis of BSPI and its fractions were investigated by appropriate differential scanning calorimetry (DSC), and Fourier transform infrared (FT-IR) spectroscopy techniques, respectively. In addition, water holding capacity (WHC) and oil holding capacity (OHC) values were determined based on the AACC method 88-04.

Differential scanning calorimetry analysis

The thermal behavior of the BSPI and its fractions was studied using a DSC system (DSC 60 Plus, Shimadzu Instruments, Japan). Approximately, 10 mg of protein bearing lyophilizates were placed in aluminum containers and heated between 20 and 160°C at a heating rate of 10°C min^{-1} (Coşkun et al., 2019).

Fourier transform infrared spectroscopy

Structural analysis of protein bearing samples was performed using an IRTracer-100 FT-IR spectrophotometer (Shimadzu, Japan) equipped with a DLATGS detector system and a MIRacle ATR module (PikeTechnologies, USA) with a resolution of 2 cm^{-1}. FT-IR absorption spectra were collected between 4000 and 650 cm^{-1} (Coşkun et al., 2019). Using the protocols defined by the instrumental manufacturer, % frequency of secondary structural elements was determined (Lee et al., 2018).

Preparation of bread samples

The raw materials necessary for gluten-free bread such as gluten-free flour (Sinangil, https://www.sinangil.com.

tr/urunler/glutensiz-unlar/glutensiz-un), instant yeast (Pakmaya), refined salt were purchased from local supermarkets. Gluten free flour consisted of corn starch, rice flour, sugar, thickeners (pectin, Xanthan gum), and leavening agents (sodium bicarbonate, sodium acid pyrophosphate). BSPI addition levels were chosen as 2% or 10% by weight. The manufacture of gluten-free bread was carried out as described by Coşkun et al. (2020). In this procedure, 300 mL of water, 300 g of gluten-free flour, 3.6 g of salt, and 12 g of instant yeast were used. First, all ingredients except BSPI were mixed and kneaded for 4 min. Then, thus formed dough was divided into smaller pieces and in each experiment, each dough piece was worked into either the control or protein fortified samples (i.e., 0%, 2%, and 10% BSPI, respectively). For this purpose, a kitchen/laboratory scale bread maker (Tefal Pain and Delices Bread Maker, TEFAL, Turkey) was utilized in mixing of control dough and BSPI. No extra heating was applied, and a 10 min mixing operation was administered using an in-built "Kneading" program. Immediately afterwards, the dough samples were fermented at room temperature (21 ± 1°C) for 45 min. The fermented dough samples were baked for 30 min at 220 and 230°C, respectively, on the lower and upper sides, using a Beko BFM 310 B oven (Beko, Arçelik A.Ş, Turkey). After baking, the samples were cooled at ambient temperature.

Texture profile analysis

Texture profile analysis (TPA) of gluten-free bread samples was performed by Tekirdağ Namık Kemal University Central Research Laboratory (NABILTEM, Tekirdağ, Turkey). TPA was performed at a test rate of 1 mm s^{-1} using a TA-XT2 Plus Texture Analyzer (Stable Micro Systems, UK). Bread samples were taken at a height of 2 cm from the center of the bread loaf and were pressed using a 36 mm radius cylindrical probe (P/236 R) for 60 s to reach 25% strain (Coşkun et al., 2020). The analysis was carried out 2 h after baking when the cooling of the bread samples was complete (Coşkun et al., 2020). Textural properties of bread samples including hardness, chewiness, gumminess, cohesion, springiness, and elasticity were determined. The necessary calculations were carried out using Texture Exponent 32 software provided by the instrument manufacturer.

Statistical analysis

The data collected in the current investigations were reported as sample means ± standard deviations based on at least triplicate experiments. Statistical significance was tested using ANOVA at 95% confidence interval and Tukey's post hoc test as necessary and appropriate. Sample mean values labeled with the same letter indicate no significant differences between the treatments.

RESULTS AND DISCUSSION

Protein determination and Osborne fractionation

The protein content of basil seeds was found to be approx. 27% ± 0.2% by the Dumas method. Prior to protein extraction, mucilage was removed by aqueous extraction and thermal hydration. Furthermore, basil oil was removed by a cold press method. Lyophilized BSPI that were prepared from cold press cakes contained approximately 95% ± 0.5% protein.

Previous data have shown that protein content of basil seeds ranged between 9% and 29% (Khaliq et al., 2017; Munir et al., 2017; Nazir et al., 2017). Consequently, current findings were comparable to previous literature. When plant foods provide more than 12% protein, they are generally considered as notable sources of protein (Calderón Bravo et al., 2021). In addition, the amino acid composition of basil seeds indicated high nutritional quality (Calderón Bravo et al., 2021; Khaliq et al., 2017; Nazir et al., 2017; Ziemichód et al., 2019). Furthermore, depending on the geographic region, the basil seeds contain approximately 15%–33% essential oil, 7% ash, 50% carbohydrates, and 7% fiber (Khaliq et al., 2017; Munir et al., 2017; Nazir et al., 2017).

In the following studies, Osborne fractionation was carried out for lyophilized BSPI and the relative distribution of its Osborne fractions were determined. Albumin, globulin, prolamin, and glutelin proteins accounted for approximately 13.5%, 16.7%, 30.0%, and 39.5% of all protein in BSPI, respectively. In order to investigate the potential of basil seed proteins in fortification of foods, further characterization was carried out.

Analysis of functional characteristics of BSPI

Determination of WHC and OHC

The WHC of the BSPI sample was 2.45 ± 0.20 g water/g protein and the OHC was 2.64 ± 0.20 g oil/g protein. WHC value of BSPI was comparable to WHC value (2.3 g/g) of chia seed protein isolates dried by spray drying method, while vacuum drying lowered the WHC value for chia proteins (Timilsena et al., 2016). The current WHC value was, however, considerably higher than that measured in Coşkun et al. (2019) by our group on black cumin protein isolates. WHC is an important parameter as it largely affects the food

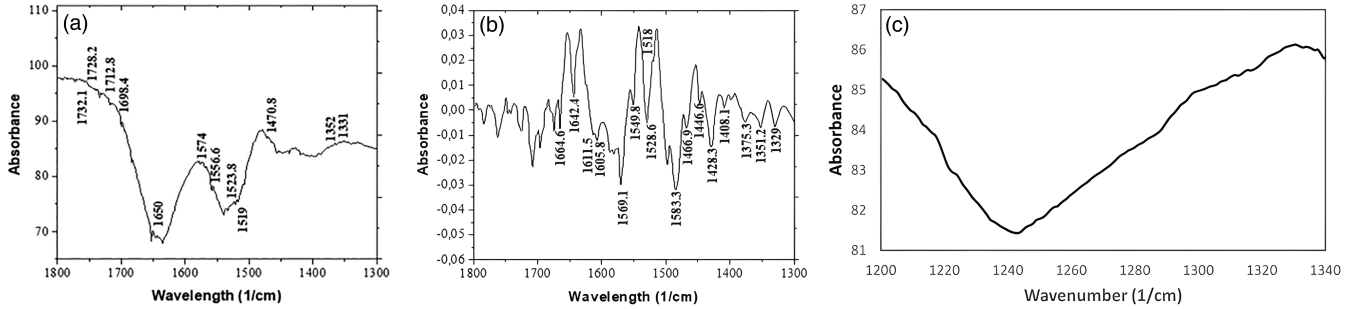

FIGURE 1 FT-IR spectrum for BSPI samples. (a) FT-IR spectrum showing amide I, and II regions, (b) Second derivatives of these spectral regions, (c) FT-IR spectrum showing amide III region. BSPI, basil seed protein isolate; FT-IR, Fourier transform infrared.

quality. Protein components with very high WHC values can dehydrate other components in a food system. Proteins with low WHC value may be more sensitive to storage humidity (Haque et al., 2016; Timilsena et al., 2016). Thus, based on the current WHC value, BSPI could be beneficial for the prevention of water loss in bread and cakes or could increase the yield of dried sausages, canned fish, and frozen products (López et al., 2019).

The OHC value of BSPI was found to be comparable to the OHC value (2.7 g/g) of the spray dried chia seed protein isolates, while OHC value of BSPI was considerably higher that of flaxseed protein isolates (1.18 g/g; López et al., 2019). Components with favorable OHC could improve the taste of foods in the mouth and also enhance the distribution of flavors throughout the food matrix. Based on the current OHC data, BSPI may potentially be used in meat and bakery formulations (Martínez-Flores et al., 2006). The non-polar or hydrophobic amino acid content in plant-derived proteins is primarily responsible for their high OHC, while OHC could be affected by protein source, size and concentration, number of apolar amino acids, processing method, and protein-lipid interactions (Aryee et al., 2018; Boye et al., 2010; Lam & Nickerson, 2013). Based on these findings, while the protein content of BSPI was reasonably high, their WHC and OHC data seem to be particularly fit for the processing of baked goods.

Analysis of structural properties of BSPI and its fractions

Fourier transform infrared spectroscopy

FT-IR spectra were evaluated in order to determine the structural properties of proteins in BSPI. First, amide I (1600–1700 cm^{-1}), amide II (1480–1575 cm^{-1}), and amide III (1400–1200 cm^{-1}) bands (Barth, 2007; Kong & Yu, 2007) were investigated (Figure 1). Some of the major peaks in the second derivative FT-IR spectrum were labeled in Figure 1b and the structural

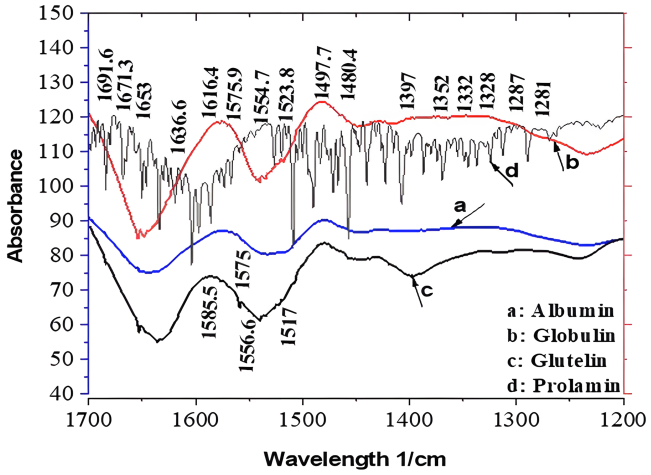

FIGURE 2 FT-IR spectra for BSPI fractions. (a) FT-IR spectrum showing amide I, II, and III regions for albumin, globulin, glutelin, and prolamin fractions. BSPI, basil seed protein isolate; FT-IR, Fourier transform infrared

elements represented by these peaks were characterized. Based on these assignments, 1673 cm^{-1} peaks indicated the presence of β-Turn structure in BSPI and 1664 cm^{-1} peaks indicated the presence of α-helical elements, respectively. Finally, peaks at 1641 and 1695 cm^{-1} detected the presence of β-sheet elements in BSPI (Figure 1b). The analysis of spectral data demonstrated that β-elements accounted for approximately 89.4% of BSPI proteins (Table 2). According to Khajehpour et al. (2006), normalized absorbance data acquired between 1000 and 1200 cm^{-1} demonstrated partial glycosylation of proteins (i.e., presence of glycoproteins). Since these peaks were minor, these findings suggested that after the removal of mucilage, glycoprotein content of BSPI was potentially negligible.

Osborne fractions of BSPI were also analyzed for their Amide I and Amide II regions in the wavenumber range of 1700–1600 cm^{-1} and 1575–1480 cm^{-1}, respectively (Figure 2). The major peaks were listed in Table 1, and structural assignments were made (Kong & Yu, 2007).

In Figure 1c, the analysis of Amide III region was plotted and peak assignments were based on Singh et al. (n.d.). The peaks in Amide III region were relatively less pronounced compared to Amide I and the absorbance values were relatively lower. The peak around 1244 cm^{-1} pointed out the presence of β-sheet elements.

As a result, β-type structures were more common than α-helix structures in Osborne fractions. Zeng et al. (2011) emphasized that the abundance of β-structures could increase the protein stability. In addition, the magnitude of peaks related to glycolysation indicated that the presence of glycoproteins in Osborne fractions was once again limited (data not shown). To support these findings, potential thermal stability of the samples was tested using a DSC technique.

DSC analysis

The thermal denaturation properties of BSPI were analyzed and the denaturation temperature was found to be approximately 110 ± 0.1°C (Figure 3). In the DSC spectra, the location and magnitude of the denaturation peak represent the thermal stability of the proteins and the corresponding enthalpy change of proteins upon denaturation. The denaturation temperature of BSPI was found to be higher than the denaturation temperatures of, for example, kaniwa (93.4°C) (Betalleluz-Pallardel et al., 2017) or chia seed proteins (97.4°C) (López et al., 2018; Timilsena et al., 2016) demonstrating pronounced thermal stability.

The enthalpy of denaturation is related to the structural characteristics of a protein (Betalleluz-Pallardel et al., 2017). The enthalpy of thermal denaturation (ΔH) for BSPI was 22.3 J g^{-1}. Compared to chia (4 J/g; López et al., 2018; Timilsena et al., 2016), amaranth 6.2 J g^{-1} (Avanza & Añón, 2007), flax 8.25 J g^{-1} (Kaushik et al., 2016) or kaniwa 1.2 J g^{-1} (Betalleluz-Pallardel et al., 2017) seed proteins, denaturation enthalpy was found to be significantly higher. This finding was also coherent with the relatively higher denaturation point. Although the denaturation enthalpies were relatively small for wheat albumins, globulins, and gliadins, comparably high enthalpies (11.47 and 14.43 J/g) were obtained in the case of glutenins which were characterized with two major peaks (León et al., 2003). These authors attributed this finding to a higher level of order in glutenin structures. It is noteworthy, however, that these authors demonstrated the influence of moisture content in DSC analysis. Denaturation enthalpies for glutenins decreased with the moisture content. Denaturation enthalpy for proteins in complex food systems are affected by protein structure, environmental characteristics, changes in protein structure and assembly during processing, and interactions with non-proteinaceous components (Li-Chan & Ma, 2002). Consequently, thermally stable proteins could preserve their technical and biological functionalities even under relatively harsh processing conditions.

Thermal denaturation properties of BSPI fractions were also analyzed. The denaturation temperatures were found to be approximately 142, 112, 93, and 107°C for albumin, globulin, glutelin, and prolamin fractions, respectively, which were relatively higher compared to various plant seed protein fractions. For example, albumin, globulin, glutelin, and prolamin fractions for chia seed proteins denatured around 103, 104, 85, and 91°C, respectively (Sandoval-Oliveros & Paredes-López, 2013). Note that all the data included in the current comparison were based on powdered protein isolates, where denaturation temperatures tend to be significantly higher than that in liquid dispersions. Denaturation temperature of all protein fractions of soybean seed except albumin (91°C) was found below 90°C. Similarly, denaturation temperatures of the seed protein fractions of winged bean were found to range between 80 and 93°C (Makeri et al., 2017). The denaturation range for wheat protein fractions were mostly between 50 and 85°C (León et al., 2003).

TABLE 2 % Frequency of secondary structure elements present in BSPI, as determined by the analysis of FT-IR spectrum data.

Secondary structure	% Frequency
α-Helix	6.4
β-Sheet	21.3
β-Turn	68.1
Random	4.2

Abbreviations: BSPI, basil seed protein isolate; FT-IR, Fourier transform infrared.

TABLE 1 Corresponding wavenumbers of major peaks for basil seed protein isolate fractions in the IR spectrum.

Wavenumber cm^{-1}	Albumin	Globulin	Glutelin	Prolamin
β-Sheet	1624, 1627, 1633, 1635, 1689, 1692, and 1696	1627, 1634, 1637, 1690, and 1694	1626, 1634, 1640, 1693, and 1698	1623, 1628, 1631, 1638, 1695, and 1691
α-Helix	1654	1656	1655	1658
β-Turn	1676, 1680, and 1684	1667, 1674, 1679, 1682, and 1686	1667, 1681, and 1685	1666, 1678, 1681, and 1685

A single endotherm peak was observed in the DSC spectra for the globulin, glutelin, and prolamin fractions (Figure 4), while various peaks were observed in the albumin spectrum. The presence of multiple peaks is attributed to the presence of proteins with varying thermostability. A single peak usually indicates that the protein isolate or fraction consists of either one protein species or several species with similar thermal stability (Betalleluz-Pallardel et al., 2017; Ruiz et al., 2016). Based on the findings, basil seeds or BSPI can be used in food systems that are exposed to intense thermal treatments due to the collective thermostability of the protein molecules in BSPI or its fractions.

The denaturation enthalpy value yields information about the amount of energy required to denature the protein structure. Enthalpy values of BSPI fractions also indicated more stable structures compared to other seed protein fractions. The enthalpy of thermal denaturation for albumin, globulin, glutelin, and prolamin fractions was approximately 79, 76, 224, and 190 J g^{-1}. In the literature, the enthalpy values of the chia, winged bean, and soy protein fractions were relatively comparable to each other (Makeri et al., 2017; Sandoval-Oliveros & Paredes-López, 2013).

Texture profile analysis

The results of the TPA for BSPI fortified gluten-free bread was summarized on Table 3, where hardness, springiness, cohesion, gumminess, chewiness, and elasticity are shown. Some examples on bread formulations examined in these analyses were demonstrated on Figure 5 as well.

As detailed above, the currently utilized commercial gluten-free flour is primarily based on corn starch, rice flour, and thickeners. In starch-based bread, water is primarily bound by the carbohydrate fraction(s). Consequently, starch-based gluten-free bread hardens much faster than gluten-bearing wheat bread. Therefore, the presence of proteins in bread could lead to significant changes on bread hardness (Ziobro et al., 2016).

TABLE 3 Average values of textural parameters of bread samples obtained by adding basil seed protein isolate (BSPI; 0%–10%) to gluten-free wheat flour at different rates. Standard deviation was <5% of the sample mean for all samples ($n = 3$).

Textural parameter	Control sample	2% BSPI	10% BSPI
Hardness (N)	30.88^{a}	33.31^{a}	28.09^{b}
Chewiness (N)	12.21^{a}	8.45^{b}	7.39^{c}
Gumminess (N)	14.04^{a}	16.43^{b}	16.17^{b}
Cohesiveness	0.46^{a}	0.49^{a}	0.58^{a}
Springiness	0.87^{a}	0.52^{b}	0.46^{b}
Elasticity	0.16^{a}	0.19^{a}	0.26^{b}

Superscripts denote whether there is statistical significance between samples.

On the day of baking, the hardness of the bread samples containing 2% BSPI were comparable to the control samples. Furthermore, lower hardness values in bread samples containing 10% BSPI were observed. The decrease in hardness was possibly due to higher WHC values of the mixture (Zorzi et al., 2020). The addition of sunflower seed protein concentrates at different concentrations also reduced the hardness of bread (Zorzi et al., 2020). In contrast, potato protein fortification of gluten-free bread generally increased the hardness of the bread, which further increased with the level of addition. Flax components lead to increased hardness in bread samples proportional to their concentration (Wirkijowska et al., 2020). Consequently, the physicochemical characteristics of added protein products were highly influential on bread structure.

Chewiness parameter is also related to the hardness attribute (Witczak et al., 2017). A significant reduction in chewiness was also detected in bread samples containing 2% and 10% BSPI, similar to the observation in hardness. While protein fortification potentially introduces the risk of increased chewiness, that was not the case with BSPI fortified bread. Any changes in chewiness were a function of concentrations for flaxseed processing by-products, while flax flour was not very influential up to 5% (Wirkijowska et al., 2020).

Gumminess is defined as the product of hardness and stickiness values and the increase in gumminess values is primarily due to the increase in hardness values in bread formulations (Šimurina et al., 2016; Ziobro et al., 2016). However, the bread samples containing 2% and 10% BSPI had comparable gumminess values as controls. Consequently, the desirability of the BSPI fortified bread remained relatively high.

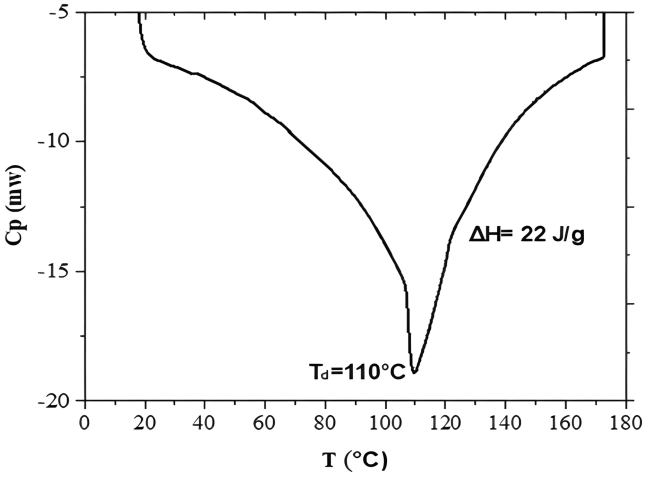

FIGURE 3 Thermal denaturation properties of BSPI lyophilizates analyzed by DSC. The heating rate was 10°C min^{-1} and the temperature range was 20–180°C. BSPI, basil seed protein isolate; DSC, differential scanning calorimetry.

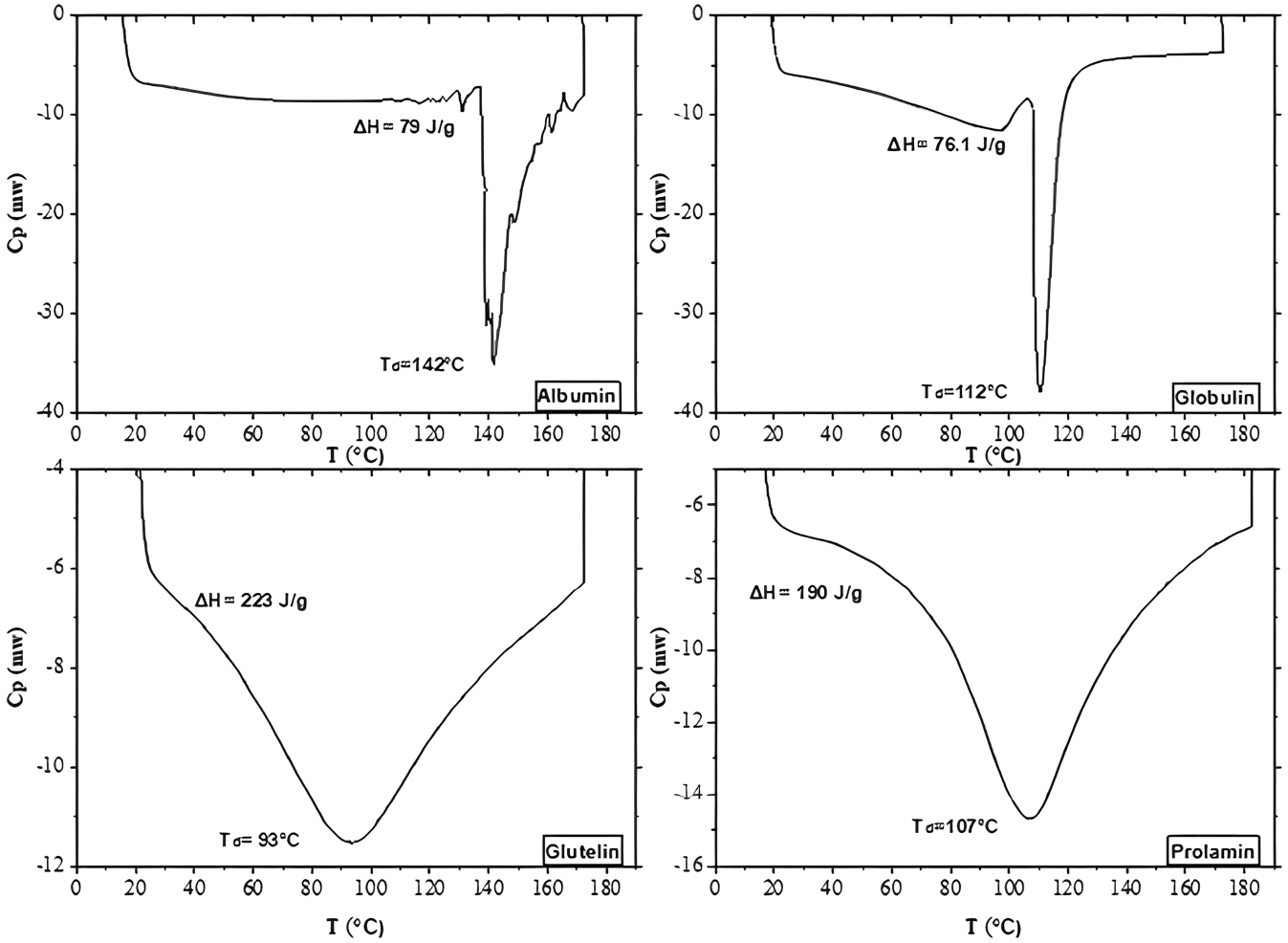

FIGURE 4 Thermal denaturation characteristics of BSPI fractions as analyzed by DSC. The heating rate was 10°C min^{-1} and the temperature range was 20–180°C. (a) Albumin, (b) globulin, (c) glutelin, and (d) prolamin fractions. BSPI, basil seed protein isolate; DSC, differential scanning calorimetry.

For baked goods, cohesion is an indicator of quality and ability to withstand deformation. Dough products with high cohesion values result in softer bread with higher specific volumes (Horstmann et al., 2017; Storck et al., 2013). Significant reduction of cohesiveness during storage results in extensive crumbling and adversely affects consumer acceptance of the bread (Crockett et al., 2011). All bread samples showed comparable cohesiveness values demonstrating that protein fortification was not influential on this parameter and BSPI fortified bread was potentially resistant to crumbling.

Springiness is a measure of the structural integrity of bread. All samples showed similar springiness, although there was a slight decrease in this value with increasing protein levels on the day of baking. Higher elasticity values were determined in the bread samples containing 2% and 10% BSPI compared with the control bread. Witczak et al. (2017) previously observed a slight decrease in the elasticity values with increasing potato protein concentrate addition levels in gluten-free bread.

Although some variations are possible due to the differences in experimental parameters, setup, or errors, most findings were comparable to the literature data. For example, Ziobro et al. (2016) reported chewiness and hardness values of gluten free bread samples in the range of approximately 0.3–6.7 N and 0.4–13.6 N, respectively. These data were mostly comparable to the current findings.

While hardness, chewiness, and elasticity values underwent changes upon BSPI fortification, gumminess, springiness, and cohesion attributes were reasonably comparable to the controls. The textural differences between 2% and 10% BSPI bearing samples were relatively small, which generated a potential for thermally stable BSPI proteins to be used in bakery products. While previous studies from our group indicated that trials with multiple plant protein isolates lead to textural problems which required corrective action to ensure acceptability (2020), BSPI bearing formulations performed relatively comparable to the gluten-free controls. Consequently, the potential of BSPI

FIGURE 5 Gluten-free bread enriched and baked with different percentages of basil seed protein isolate (BSPI; 0%–10%). From left to right: Control bread, 2%, or 10% BSPI enriched bread. The increase in BSPI was proportional to the darker bread color.

remains to be further investigated in various bakery applications.

CONCLUSIONS

Due to the increasing global demand for protein, continuous efforts are carried out to utilize proteins from a variety of unconventional sources. Therefore, a better understanding of the structure–functionality relationship for novel protein products could significantly improve the quality of food products upon protein fortification.

Although basil is widely distributed globally, data on basil seed proteins are mostly limited. In this study, BSPI were prepared and evaluated in terms of their physico-chemical properties, and technical functionalities. To further analyze the structural attributes, basil seed proteins were separated into their Osborne fractions. Finally, textural properties of the BSPI fortified gluten-free bread were investigated.

Denaturation temperature and enthalpy values obtained by DSC demonstrated that BSPI was stable up to 109°C. In addition, albumin, globulin, and prolamin fractions were characterized with denaturation temperatures above 100°C. Consequently, BSPI and its fractions may be utilized in high temperature food manufacture processes such as baking (Petruzzi et al., 2017). Denaturation is likely to generate stronger protein–protein interactions, which in turn reduces the strength of protein–water (e.g., syneresis in yogurt products) or protein–starch (in baked products) interactions. Consequently, thermally stable proteins are more likely to allow fine-tuning of food structures during industrial processing. While partial denaturation may lead to enhanced functionality in various food systems, in many cases complete denaturation could lead to reduced functionality (Damodaran, 2005). BSPI samples also demonstrated significant WHC and OHC values. While BSPI and its fractions contained limited glycosylation, α-type and β-type structural elements were identified. In particular, beta motifs were thought to be dominant potentially leading to enhanced thermal stability. This observation was particularly suitable for the fraction with the highest denaturation temperature (i.e., albumin).

Since Osborne fractionation is based on solubility differences, in this particular case, the major issue is their contribution to aqueous solubility/dispersibility and WHC, while in other industrial products, expectations and observations could be fundamentally different. In the current study, WHC value of BSPI was considerably high, and BSPI could be conveniently dispersed in the dough samples. We believe that predominantly hydrophilic BSPI fractions were instrumental for this observation.

BSPI fortification of gluten-free bread potentially enhanced or preserved the technological characteristics of bread samples. BSPI could serve as a glycemic index reducing agent in gluten-free flours, which are typically characterized with elevated concentrations of carbohydrates. We anticipate that basil proteins may be employed in fortification of bread in countries where basil seeds are produced in high quantities and are relatively affordable. Under such circumstances, BSPI fortification in gluten-free bread would not only improve nutritional and technical quality, but also could serve as a cost-effective and high-quality protein source from local and sustainable sources.

AUTHOR CONTRIBUTIONS

Mohammad Rashid was responsible for methodology, formal analysis, investigation, data curation, writing of the first draft. İbrahim Gülseren was responsible for conceptualization, methodology, resources, review and

editing, supervision, and project administration. All authors have read and agreed to the published version of the manuscript.

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CONFLICT OF INTEREST STATEMENT

The authors declare that they have no conflict of interest.

ETHICS STATEMENT

No human or animal subjects were used in this research.

ORCID

İbrahim Gülseren https://orcid.org/0000-0002-7339-1159

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