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PHYSICAL PROPERTIES OF PROTEIN EXTRACT POWDER FROM STALE BREAD AND EVALUATION OF ITS USE IN WHEAT BREAD CONTAINING CHICKPEA FLOUR

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ABSTRACT
In this study, it was aimed to investigate the physical properties of protein extract powder from stale bread and its potential use in chickpea flour added wheat bread formulations. Chickpea flour was added into wheat flour at a 2:3 ratio, and the protein extract at 6.5 % and 13 % of total flour mix (w/w). The moisture content and water activity of the powdered protein extract were 5.55% and 0.58, respectively. The bulk density and tapped density were 830 kg.m^{-3} and 910 kg.m^{-3}, respectively. Hausner ratio was measured as 1.10, which indicates free-flowing property. Increasing the addition of protein extract from 6.5% to 13% resulted in increased loaf volume from around 283 mL to 307 mL per loaf.
Keywords: Loaf volume, chickpea flour, FTIR, protein powder

BAYAT EKMEKTEN ELDE EDİLEN PROTEİN EKSTRAKT TOZUNUN FİZİKSEL ÖZELLİKLERİ VE NOHUT UNU İÇEREN BUĞDAY EKMEĞİNDE KULLANIMININ DEĞERLENDİRİLMESİ

ÖZ
Bu çalışmada, bayat ekmekten elde edilen protein ekstraktı tozunun fiziksel özelliklerinin ve nohut unu katkılı buğday ekmeği formülasyonlarında potansiyel kullanımının araştırılması amaçlanmıştır. Nohut unu, buğday ununa 2:3 oranında ve protein ekstraktı tozu toplam un karışımının ağırlıkça % 6.5 ve 13'ü oranında ilave edilmiştir. Toz protein ekstraktının nem içeriği ve su aktivitesi sırasıyla % 5.55 ve 0.58 olarak bulunmuştur. Yığın yoğunluğu ve sıkıştırılmış yoğunluğu sırasıyla 830 kg.m-3 ve 910 kg.m-3 olarak hesaplanmıştır. Hausner oranı 1.10 olarak ölçülmüştür ki bu da serbest akış özelliğine işaret etmektedir. Protein ekstraktı ilavesinin %6.5'ten 13'e arttırılması, ekmek hacminin ekmek başına yaklaşık 283 mL'den 307 mL'ye artmasıyla sonuçlandığı tespit edilmiştir.
Anahtar kelimeler: Ekmek hacmi, nohut unu, FTIR, protein tozu

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INTRODUCTION

Bread consumption has increased steadily in many parts of the developing world due to changing dietary habits and ever-evolving populations (Mtelisi Dube et al. 2020). In order to meet the growing consumers' expectations, it is necessary to improve the quality of the bread products. In addition, it is imperative to reduce the waste generated by throwing stale bread into the bin (Doğan et al. 2012).

The estimated production volume of bread is around 100 million tons per year, and around 10% of total production is discarded worldwide due to poor quality, microbial spoilage and staling (Nionelli et al. 2020). Although bread is a staple food, it loses its desired properties due to staleness. Depending on the staleness of the bread, loss of aroma, loss of softness, and crumbling occur in the bread (Choi et al. 2008). While stale bread is still fit for consumption, the loss of texture and taste reduces consumer acceptance and poor sensory quality. Therefore, the stale bread is used as animal feed or thrown into the environment as waste (Doğan et al. 2012). However, the protein fraction of stale bread may serve as a suitable ingredient in the dough formulations to improve the protein content of composite flour mixtures.

In general, the protein content of bread is around 8-13% (Turkomp 2019). Gluten is responsible for the extensibility and elasticity of the dough, and its functionality is closely related to processing quality of wheat dough (Tronsmo et al. 2003). Gluten can be added to weak wheat flours or flour mixtures to improve technological properties (i.e., dough processability and loaf volume). Bread made from flour containing a sufficient amount of gluten has a higher volume and slower staling rate than bread made with gluten-poor flour. Therefore, the protein fraction of stale bread can serve as a suitable ingredient for dough formulations.

Various studies have been conducted to extract protein fractions from various sources and used in dough formulations (Begum et al. 2011; Dua et al. 2009; Jiamyangyuen et al. 2005; Wieser 1998). However, no information was found in the literature about protein extraction from stale bread and the evaluation of its functionality.

Since refined wheat flour lacks some dietary substances such as dietary fiber, and some essential amino acids (i.e., threonine and lysine) (Boye et al. 2010), enrichment with natural sources of functional compounds would be an effective means to improve its nutritional quality. Chickpea (*Cicer arietinum* L.) flour has high-quality protein containing well-balanced amino acid composition, dietary fibers, minerals, and other bioactive compounds and is a valuable ingredient for increasing the nutritional value of bread and bakery products (Gaur et al. 2015; Gobbetti et al. 2020; Man et al. 2015). Even though chickpea flour is a good source of protein, there is no gluten in its structure which causes some adverse effects on bread quality. Guardado-Félix et al. (2020) and Mohammed et al. (2012) reported that the addition of more than 10% of chickpea flour decreased bread volume and increased crumb firmness.

Taking into account the explanations given above, it was aimed to extract the protein fraction from stale bread, characterize its powder properties, and evaluate its potential to improve the quality of chickpea flour added wheat bread.

MATERIAL and METHODS

Material

The bread samples used in the tests were purchased from a local grocery store and kept at room temperature for two days to become stale. The chemicals and reagents were obtained from Sigma Aldrich (Darmstadt, Germany).

Methods

Sample preparation

Stale bread was cut into small pieces and placed in an oven to dry at 80°C for about four h until the moisture content was reduced to approximately 10%. After drying, the bread pieces were grounded into powder using a coffee grinder (Sinbo SCM-2934).

Protein extraction

The method reported by Joshi et al. (2011) was used to extract protein from stale bread. The dried and grounded stale bread samples were mixed with distilled water at a ratio of 1:15. The pH of the resulting mixture was adjusted to 9.50 by adding 1.0 M NaOH solution. The mixture was stirred at 750 rpm with the aid of a magnetic stirrer for 60 min at room temperature. The mixture was then centrifuged at 12000 rpm for 30 min. The pH of the supernatant was adjusted to 4.5 with 1 M HCl. After the second centrifugation under the same conditions, the liquid phase was removed, and the pellet was collected. The pellet was placed in a glass petri dish and dried in an incubator at 35 °C for approximately 36 h.

Evaluation of protein yield

In order to evaluate the protein yield, the weight of the bread used in protein extraction was weighed, then the amount of protein obtained was given as % (wt/wt).

Determination of physicochemical properties of protein powder

Protein content determination

Protein content was measured by Bradford Assay method (Bradford 1976). To prepare 1 L Bradford reagent, 0.1 g of Coomassie Brilliant Blue G-250 was dissolved in 95% ethanol, and 100 mL of 85% phosphoric acid was added, and the final volume was made up to 1 L with distilled water. The Bradford reagent prepared was filtered on filter paper. 1:1 ratio (protein powder: distilled water) of dispersion was prepared and passed through filter paper. Five-mL of Bradford reagent was added to 100 μL of filtrate and left for 5 min. Absorbance values at 595 nm were recorded, and the percent protein content was calculated using the standard calibration curve. The analysis was repeated three times. Bovine serum albumin (BSA) was used to prepare the standard calibration curve. BSA standard solution (1 g/mL) was diluted to 10, 20, 40, 60, and 100 μg/mL with distilled water and a standard calibration curve was created by measuring the absorbance values using a UV-spectrophotometer (Shimadzu UV-1280, Kyoto, Japan).

Water activity determination

The protein powder was placed in the Novasina LabSwift water activity meter (Novasina, Lachen, Switzerland), and the measurement was performed according to the manufacturer's instructions.

Moisture Determination

The infrared moisture analyzer (Radwag, Radom, Poland) was employed for moisture analysis. Approximately 1 g of sample was placed on the weighing plate. The method was conducted based on the manufacturer's instructions.

Determination of powder properties

Bulk and tapped densities

To determine the bulk density of the protein powder, 2 g of sample was transferred into a 10 mL graduated cylinder, and its volume was recorded. Bulk density was calculated by dividing the mass by volume ($kg.m^{-3}$). Then 150 taps (compression) were performed using a glass rod at a constant speed on the powder sample while in the graduated cylinder. The tapped density was calculated by dividing the mass by the volume of the sample after the taps (Ermis et al. 2018). The flow property of protein powder was evaluated by the Hausner ratio (HR) using Eq. 1 (Hausner 1967) and Table 1.

$$HR = \varrho_t / \varrho_b \quad (1)$$

Where ϱ_t is tapped density and ϱ_b is bulk density Table 1

Table 1. Classification of powder flow based on Hausner Ratio (Ermis et al. 2018)

Powder Flow Behavior	Hausner ratio
Easy	1.00-1.11
Good	1.12-1.18
Satisfactory	1.19-1.25
Poor	1.26-1.34
Very poor	1.35-1.45

Wettability

The powder sample (1 g) was left on the surface of a beaker filled with 400 mL of distilled water. The time required for the powder sample to submerge to the bottom of the beaker was recorded (Seth et al. 2016).

FTIR analysis

FTIR spectrum of extracted protein powder was obtained to compare its chemical structure with gluten powder. The crystalline region of the ATR apparatus was cleaned with acetone prior to obtaining the background spectrum. Then, the powder sample was placed on the crystalline chamber of the device, and the spectrum was obtained between 4000-400 cm^{-1} by using an ATR-FTIR (Attenuated Total Reflectance-Fourier Transform Infrared Spectroscopy) instrument (Shimadzu IRTracer-100, Kyoto, Japan).

Bread production

For the preparation of bread samples, the dough formulation reported by Begum *et al.* (2011) was used in this study with slight modifications. Protein extracts were added into dough formulation at two different ratios (6.5 and 13%) based on the total amount of flour mixture consisting of 60% wheat flour and 40% chickpea flour (Table 2). As the first step, instant dry yeast was dissolved in water, and then salt was added before transferring into the dough mixer. Other ingredients were transferred into the mixer, and the remaining water was added. The dough was mixed for the first two min at low speed and the next 10 minutes at medium speed. The dough was rested at room temperature for 45 min. The dough is then divided into 100 g pieces, rounded, and proofed at 40 °C for 45 min. The proofed dough was baked in the preheated oven for 20 min at 200 °C. Bread volume was determined based on official methods of AACC 10-10.03 (AACC 2000).

Table 2. Ingredients used for preparing bread

Ingredients (g/100 g)	Control 1	Control 2	A	B
Wheat flour	65	39	36.6	34.2
Chickpea flour	0	26	24.4	22.8
Water	33	33	33	33
Active dry yeast powder	1	1	1	1
Salt	1	1	1	1
Protein extract	0	0	4	8

Statistical analysis

The experiments were conducted in triplicate, and the mean values with standard deviations were calculated. Minitab 17 (Minitab Inc., Pennsylvania USA) was used to perform the statistical evaluations using one-way analysis of variance (ANOVA) and Tukey's multiple comparison test ($P<0.05$).

RESULTS and DISCUSSION

Evaluation of protein yield

The yield of protein extract from stale bread was recorded as 1 g protein per 100 g of stale bread (Figure 1). Since the protein content of wheat bread is around 10 %, approximately 10% of total protein (w/w) could be extracted. The low yield might be due to the alteration of solubility behavior and the isoelectric point of protein fractions denatured during baking. Therefore, it was not possible to precipitate all the protein fractions at an isoelectric point of 4.5.

Figure 1. View of extracted protein from stale bread

Powder properties

The water activity, moisture content, bulk density, tapped density, Hausner ratio, and the wettability properties were determined as 0.58, 5.55 %, 830 kg m^{-3}, 910 kg m^{-3}, 1.10, and 816 s, respectively. Moisture content and water activity are parameters that affect powder behavior, such as powder flow and storage stability. Water activity is more related to the food's physical, chemical, and biological properties than its moisture content. It is an important parameter that affects various food properties such as texture, color, and microbial growth. Generally, powders stored at low a_w values and low temperatures retain their free-flowing properties, while stickiness and caking may occur at high a_w and temperature values (García-Tejeda et al. 2019).

Moisture content and water activity values were found at safe levels to ensure storage and microbial stability. In order to ensure microbial stability in foods, the water activity value must be below 0.6. Also, moisture content should be below 10% to preserve the food for a more extended period while preserving its quality properties (Dirim and Talih 2018).

The wettability of the protein powder was measured as 816 s (13.6 min) in this study. The wettability is an important physical property to be used in bread making due to its effect on dough development (Blancher et al. 2005). The wettability time gives information about the hydrophilic property of the powder material (Dirim and Talih 2018). A study observed that the wettability time of milk protein isolate was more than one h (Ji et al. 2016). According to Ji et al. (2016), powders showing wettability greater than 120 seconds are considered non-wettable. Thus, it can be stated that the protein powder obtained from stale bread by the alkali extraction-isoelectric precipitation method is non-wettable.

Bulk density is a parameter that plays a role in transporting and packaging powder products. High bulk density is a desired feature as it will provide lower packaging and transportation costs (Dirim and Talih 2018). The ratio between tapped density and bulk density is determined as the Hausner ratio. HR was calculated as 1.10, indicating easy flow behavior (Table 1).

FTIR properties

The chemical structure of the protein extract was examined by a FTIR spectrometer (Figure 2). Gluten standard was used as the reference material. The robust transmissions at 1653 cm^{-1} at 1748 cm^{-1} show C=N and C=O bonds, respectively. Peaks at 2800-2900 cm^{-1} region are assigned to a symmetrical and asymmetrical stretching of CH_2 and CH_3 groups. An Amide I band and an N-H intense stretching can be found at 2924 cm^{-1}. C-H bonds at 2855 cm^{-1} clearly show us the presence of protein fractions. Amir *et al.* (2013) observed two main absorption bands, amide I and amide II, at approximately 1660 cm^{-1} and 1550 cm^{-1}, respectively. The amide I peak centered around 1652 cm^{-1}in Figure 2 was similar to the findings of Lin *et al.* (2021) and Nawrocka *et al.* (2018). The FTIR data revealed differences in amide I and amide II peak intensities of extracted protein and gluten. Similarly, the peak intensities within the 750-1250 cm^{-1} region show differences in chemical properties. These differences might be attributed to the denaturation of proteins during baking and the existence of some impurities such as polysaccharides in extracted protein (Nawrocka et al. 2018).

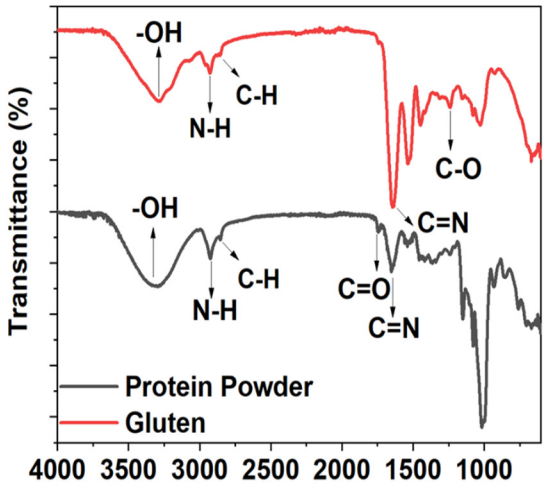

Figure 2. FTIR spectra of the extracted protein and gluten standard

Evaluation of loaf volume

The loaf volume is an essential physical attribute in assessing bread quality. It is affected by the chemical structure of flour (i.e., gluten quality and content, amount of damaged starch) (Araki et al. 2009) and conditions applied during processing steps such as milling, mixing, kneading, fermentation, and baking. Assessment of bread loaf volume is a helpful way to determine the differences in the functionality of extracted proteins (Jiamyangyuen et al. 2005). The obtained protein powder was added to the bread formulations at 6.5% and 13% concentrations (based on total flour weight). The loaf volumes are reported in Table 3. The volume of Control 1 (prepared using 100% wheat flour) and Control 2 (prepared using 60% wheat flour+40% chickpea flour mixture) bread samples were measured as 307.33±2.0 and 282.67±5.33 mL, respectively. The loaf volume of Control 2 bread was smaller when compared to those of bread samples A and B. Increasing the protein added resulted in improved loaf volume by approximately 10 and 20 % for A and B, respectively (Figure 3). This increase could be attributed to the addition of protein powder extracted from stale bread.

Table 3. The volume of bread samples

Sample	Volume (mL)
Control 1	307.33±2.00c
Control 2	282.67±5.33d
A	312.67±3.33b
B	342.00±4.67a

Control 1: 100 % wheat flour
Control 2: 60 % wheat flour, 40 % chickpea flour
A: 60% wheat flour + 40% chickpea flour + 6.5% protein,
B: 60% wheat flour + 40% chickpea flour + 13% protein
Values shown with different letters in the same column are statistically different ($P < 0.05$)

Figure 3. View of bread samples. Control 1: 100% wheat flour, Control 2: 60% wheat flour+40% chickpea flour, A: 60% wheat flour+40% chickpea flour+6.5% extracted protein, B: 60% wheat flour+40% chickpea flour+ 13% extracted protein

CONCLUSION

In this study, protein extraction was made from stale bread by the isoelectric precipitation method, and it was aimed to increase the bread volume by adding into the mixtures of chickpea flour and wheat flour. Around 10 % of the total protein could be extracted from stale bread and dried to produce protein powder. The powder had around 10 % moisture and showed free-flowing behavior. FTIR analysis of protein extract exhibited similar spectra of the gluten standard. Adding 8 g extracted protein caused around 10% and 20% volume increase in the wheat bread and chickpea flour added wheat bread, respectively. The data obtained in this study are promising and provide a scientific basis for further studies. Future studies should focus on improving protein extraction yield from stale bread by using different extraction methods and optimization of extraction conditions should be studied.

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CONFLICT OF INTEREST
The authors declare no conflict of interest.

AUTHORS CONTRIBUTIONS
Sebahat Şişman performed investigation, formal analysis and writing–original draft. Büşra Yağcı helped in investigation, and formal analysis. Ertan Ermiş was supervisor and guided the project, performed final writing-review & editing.

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