Pilot Plant Scale Manufacture of Bread Enriched with Seed Protein Concentrates

Özgenur Coşkun1,2,a, Halime Pehlivanoğlu3,b, İbrahim Gülsener1,4,c,*

1Department of Food Engineering, İstanbul S. Zaim University (İZÜ), Halkalı Campus, 34303 Kucukcekmece, İstanbul, Turkey
2Department of Food Science, Aarhus University, Denmark
3Department of Food Hygiene and Technology, Faculty of Veterinary Sciences, Namık Kemal University (NKÜ),59030 Tekirdağ, Turkey
4İZÜ Food and Agricultural Research Center (GTAUM), Kucukcekmece, İstanbul, Turkey
*aCorresponding author

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ABSTRACT

For many seeds, cold press technology generates higher quantities of cakes than seed oils, which are concentrated in proteins. Valorization of the cakes could offer a viable strategy to manufacture protein fortified foods with comparable characteristics as the conventional products. Here, black cumin, grape seed and pumpkin seed protein concentrates were prepared based on an alkaline extraction-isolectric precipitation technique. The influence of protein concentrate addition on the flour, dough and bread characteristics were investigated for textural profile, gluten quality and visual characteristics including color attributes. While the interactions between gluten and seed proteins were mostly weak, some of the physicochemical attributes differed significantly. In terms of volume and visual characteristics, pumpkin seed protein concentrates enriched bread demonstrated similar characteristics as the controls, while black cumin or grape seed protein concentrate enriched wheat flours were more resistant and less extensible than the controls. Similarities and differences between controls and protein enriched gluten-free or gluten-bearing bread were discussed.

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Tohum Protein Konsantrlerince Zenginleştirilmiş Pilot Tesis Ölçekli Ekmek Üretimi

MAKALE BİLGİSİ

ÖZ

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Anahtar Kelimeler:
Soğuk pres posası
Cörek otu
Üzüm çekirdeği
Kabak çekirdeği
Proteine zenginleştirme

Birçoğu tohumun soğuk pres tehnolojisi ile işlenmesi sürecinde, üreten yağdan daha fazla miktarlarda proteine zengin posa (pres kekleri) açığa çıkmaktadır. Posanın değerlendirilmesi, proteinle zenginleştirilmiş ve diğer özellikleri konvansiyonel ürünlerle benzenyen gıdaların üretilmesi için uygun bir strateji sunmaktadır. Bu çalışmada çörek otu, üzüm çekirdeği ve kabak çekirdeği protein konsantrleri alkali ekstraksiyon-izoelektrik çökeltme tekniğine uygun olarak üretilmiştir. Protein konsantrleri eklenenin, hamur ve ekmek özellikle artışı tekrar profili, gluten kalitesi ve renk özellikleri de içermek üzere gőrsel özellikler açısından incelenmiştir. Gluten ve tohum proteinleri arasındaki etkileşimler çokluhalık zaryatık, bazı fizikokimyasal özelliklerini değiştirliği bulgulanmıştır. Hacim ve gőrsel özellikler açısından, kabak çekirdeği protein konsantrisi ile zenginleştirilmiş ekmek, kontrol grubu ile benzer özellikleri gösterirken, çörek otu veya üzüm çekirdeği protein konsantrisi ile zenginleştirilmiş buďday unlar, kontrollere göre daha fazla direnç gösterdikleri ve daha düşük uyayabilirlik değerlerine sahip olduklarını bulmuştur. Kontroller ve proteinle zenginleştirilmiş glutensiz veya gluten içeren ekmekler arasındaki benzerlikler ve farklılıklar tartışılmıştır.
Introduction

Bread is a staple food that is consumed globally and most often, wheat is utilized in its production. In most industrial bread formulations, bran is removed from the wheat flour due to ease of processing, while wheat flour becomes poorer in terms of essential amino acids (Delcour and Hoseney, 2010). Consequently, protein enrichment of bread is an active area of research (Stelten et al., 2014). Proteins could enhance technical attributes of processed foods including textural and flavor related characteristics as well as its storage stability. Protein based ingredients will especially be practical, when they can be directly incorporated into the formulations, for example using their powdered forms (Storck et al., 2013).

Press meals or cakes are among the primary by-products of vegetable oil processing. Especially in the context of cold press technology, considerable quantities of cakes form. In many cases, such by-product streams (i.e., press cakes) are characterized by their technically and biologically functional molecule content and their valorization could lead approximately to zero waste and sustainable, circular economic strategies (Drożłowska et al., 2020). Most seeds have significant concentrations of protein prior to processing. Removal of seed oil further concentrates the protein molecules in the cakes. Consequently, valorization of press cakes seems to be a viable strategy in the manufacture of protein fortified food products (Coşkun et al., 2020).

Black cumin (BC) (Nigella sativa) is a valuable and annually flowering medicinal plant from Ranunculaceae family that is native to the Eastern Mediterranean countries, Southern Europe and Asia Minor (Baydar, 2009; Baytop, 1999). Black cumin seeds contain approx. 21% protein, whereas its corresponding cold press cake could easily account for >30% protein (Coşkun et al., 2019). Previously pumpkin seed ingredients including pumpkin seed (PS) proteins have been utilized in both food formulations and nutritional supplements thanks to their health-promotion potential (Bucko et al., 2015; El-Soukkary, 2001). Based on simple aqueous extraction techniques, it is possible to generate pumpkin seed protein concentrates with high protein content (>80%) (Coşkun and Gülsener, 2020). Grape (Vitis vinifera L.) seeds (GS) constitute another abundant source of seed protein, since approx. 69 million tons per year of grapes are produced globally (FAO/STAT, 2011). The seeds account for approximately 2-3% of the harvest and the protein content in the seeds is approx. 10-13% (Fantozzi, 1981).

While valorization of cold press cakes could offer a viable strategy to manufacture protein fortified bread, the characteristics of the final products must be comparable to that of the conventional products in terms of textural profile, gluten quality and visual characteristics among other attributes.

Farinograph and extensograph measurements are generally used to determine the bread making properties of bread flour and structural attributes of the dough. Farinograph analysis yields information about the characteristics of kneading dough and baking bread. The ability of the dough to retain carbon dioxide during fermentation is related to extensibility and resistance to extension parameters measured in farinographic analysis, which in turn, are important for bread making properties of the flour. Extensograms yield information about the overall quality of the flour and its response to additives (Delcour and Hoseney, 2010). For example, Ziobro et al. (2013) previously reported about the strong influence of plant protein additives on dough properties.

In our previous studies, cold press cakes of BC, PS and GS were utilized in the manufacture of gluten-free bread formulations (Coşkun et al., 2020). It was found that addition of protein concentrates (PC) significantly increased firmness and decreased springiness of the crumb with the exception of pumpkin seed protein concentrate (PSPC) samples, while further water inclusion enabled the correction of the firmness attribute.

In this study, a further effort was made to valorize cold press cakes of BC, PS and GS to generate protein concentrates (PC) (i.e., BCPC, PSPC and GSPC, respectively), and utilize them in protein enrichment of gluten bearing bread products. The influence of protein enrichment on the textural properties of the flour, dough and bread samples was investigated and the findings were compared to the earlier findings on gluten-free bread products regarding protein–protein and protein–water interactions. The current techniques are highly adaptable to industrial bread manufacture practices and could be exploited as a strategy to valorize vegetable oil manufacturing by-products including but not limited to that of currently analyzed seeds.

Material and Methods

Materials

Cold press cakes of black cumin, pumpkin, and grape seed meals were generously donated by Oneva (Neva Foods Ltd., İstanbul, Turkey), a local manufacturer of cold press oils. The material for dough and bread making consisted of wheat flour, salt and compressed yeast (Marmara Maya, İstanbul, Turkey) were purchased from a local store. All chemicals used were of reagent grade and purchased from Sigma-Aldrich Corp (Schnelldorf, Germany).

Methods

Preparation of Seed Protein Concentrates via Alkali Extraction-Isoelectric Precipitation Method (AE-IP)

Protein concentrate preparation technique was based on the solubilization of protein molecules at basic pH, which was followed by the isoelectric precipitation at acidic pH values (Boye et al., 2010). Briefly, 50 g of cold press cake was dispersed in water (1:15, w/v) and medium pH was adjusted to pH 9.5 using 1.0 N NaOH. The dispersions were stirred at 500 rpm for 1 h at ambient temperature (22±1°C). Immediately afterwards, the dispersions were centrifuged at a rate of 4200 ×g (Mixtasel-BL centrifuge, Spain) for 30 min. The supernatant containing the solubilized proteins was collected and medium pH was adjusted to pH 4.5 using 1.0 N HCl to induce isoelectric precipitation. To ensure the complete separation of precipitating proteins, the supernatant was again centrifuged at 4200×g (30 min). The pellet was collected, frozen at −20°C and lyophilized using a Teknosem TRS.
Bread preparation and analysis were carried out based on the internal procedures of the supporting company (Polen Foods, Istanbul, Turkey). The composition of each and every bread recipe was listed on Table 1. For bread production, 430 g water, 200 g ice, 1000 g wheat flour, 15 g salt and 25 g compressed yeast were used. The protein content was enhanced by 1.5% (w/w) in all cases. Firstly, all ingredients except salt and yeast were added to the mixing vessel (DIOSNA - Dierks & Söhne GmbH, D-49074, Germany) and mixed for 4 minutes. Then other ingredients were added to the mixing vessel and bread dough was made. The doughs were rolled and shaped (Ekmasan EK-37, Turkey) and fermentation was carried out using a Juniorlev cabinet (Tecnomac, Italy) for 3 different fermentation durations (80, 110, or 140 min). In this context, a total of 4 different types of bread utilizing 3 different protein concentrates and a control sample and 3 different fermentation times were studied. Baking was carried out for 30 min using an industrial oven (Wiesheu Wolfen GmbH, Germany). The bottom and top were baked at 220°C and 230°C, respectively. Once baking was complete, the samples were removed from the oven, and cooled at ambient temperature (21°C±1).

**Wheat Flour Analysis**

Falling number (ICC method (1995) using Perten, 1500, Sweden), fungal falling number (Perten, FN 1900, 1995), sedimentation, delayed sedimentation (ICC method 116/1, 40 cycle/minutes, Erkaya, Zeleny 120, Turkey), wet gluten and gluten index (ICC method 137-1 (2000) using Perten GM 2200, Sweden) of control wheat flour and wheat flour enriched with protein concentrates were determined based on the listed methods (ICC, 1995, 2000, 2018; Perten, 1995).

**Determination of Dough Rheology**

Rheological properties of dough samples were based on farinograph and extensograph measurements. Farkinograph analysis was based on AACC method 54.21 using Brabender Farkinograph-E 810114, whereas extensograph analysis utilized AACC method 54.10 using Brabender Extensograph-E 860702, Germany (AACC, 2000a, b).

**Texture Profile Analysis**

Texture profile analysis (TPA) of bread crumbs was performed using TA-XT2plus texture analyzer (Stable Micro Systems, England), according to standard program, at the test speed rate 1 mm/s. Bread crumb sample, taken from the center of the loaf with a height of 2 cm was pressed to reac...
Table 2. Analytical quality parameters of protein-fortified flours. F-BCPC: Wheat flour enriched with black cumin protein concentrate; F-GSPC: Wheat flour enriched with grape seed protein concentrate; F-PSPC: Wheat flour enriched with pumpkin seed protein concentrate. One-way ANOVA with post hoc Tukey tests were carried out to compare groups and establish statistical significance (P<0.05). In all cases, at least three replicates were analyzed.

| Samples          | Wet gluten (%) | Gluten index | Sedimentation | Delayed sedimentation | Falling number | Fungal falling number |
|------------------|----------------|--------------|---------------|-----------------------|----------------|-----------------------|
| Control          | 25.2±0.14^b   | 99±0.07^a    | 31±0.07^b     | 37.5±0.70^a           | 416.5±23^a     | 922±0.00^a            |
| F-BCPC           | 24.9±0.14^b   | 98±2.12      | 33±0.00^a     | 36.5±0.70^b           | 399±7.1^b      | 913±4.94^a            |
| F-GSPC           | 27.9±0.21^b   | 96±1.41      | 26±0.00^b     | 32.5±0.70^b           | 389±8.5^b      | 824±5.65^b            |
| F-PSPC           | 25.1±0.07^a   | 98±1.41      | 31±0.00^b     | 37.5±0.70^a           | 378±5.7^a      | 906±7.77^a            |

Table 3. Extensograph parameters of dough samples prepared from protein-fortified flours. D- BCPC: Dough prepared from black cumin protein concentrate enriched wheat flour; D-GSPC: Dough prepared from grape seed protein concentrate enriched wheat flour; D-PSPC: Dough prepared from pumpkin seed protein concentrate enriched wheat flour. One-way ANOVA with post hoc Tukey tests were carried out to compare groups and establish statistical significance (P<0.05). In all cases, at least three replicates were analyzed.

| Dough resting time | Sample     | Energy (cm²) | Resistance to extension (BU) | Extension (mm) | Maximum (BU) |
|--------------------|------------|--------------|------------------------------|----------------|--------------|
| 45 minutes         | Control    | 70±3.2^a     | 302±5.9^b                   | 134±4.2^a      | 375±10.01^a  |
|                    | D-BCPC     | 72±4.1^a     | 344±6.3^b                   | 128±11.1^b     | 411±5.9^b    |
|                    | D-GSPC     | 85±5.6^b     | 331±7.2^c                   | 143±3.2^c      | 448±8.9^c    |
|                    | D-PSPC     | 73±7.1^d     | 274±10.2^d                  | 149±6.5^d      | 364±15.6^d   |
| 90 minutes         | Control    | 100±4.5^a    | 484±9.5^a                   | 129±4.5^b      | 554±25.3^a   |
|                    | D-BCPC     | 91±5.3^a     | 540±4.8^b                   | 113±1.2^c      | 620±30.2^b   |
|                    | D-GSPC     | 101±6.2^b    | 572±3.6^d                   | 116±10.3^d     | 692±24.8^d   |
|                    | D-PSPC     | 87±1.3^c     | 413±4.2^d                   | 128±4.6^d      | 508±41.3^d   |
| 135 minutes        | Control    | 86±2.6^a     | 473±7.9^e                   | 117±5.3^b      | 542±12.35^e  |
|                    | D-BCPC     | 100±6.1^b    | 544±8.1^b                   | 121±8.2^d      | 637±26.3^b   |
|                    | D-GSPC     | 93±3.2^c     | 576±11.6^d                  | 111±9.6^d      | 662±45.6^d   |
|                    | D-PSPC     | 78±4.7^d     | 389±13.9^e                  | 127±4.6^c      | 476±30.2^d   |

Falling number and fungal falling number measurements indicated that in most cases, a relatively higher -amylase activity was observed in the protein enriched samples, possibly due to the -amylase content of the protein concentrates (Table 2). In general, gluten related characteristics were relatively unaltered, whereas minor changes could be expected in the starch related attributes due to increased -amylase activity. Physical changes in starch or gluten characteristics of dough samples were recently shown to affect their gelatinization behavior (Paulik et al., 2019). While the interactions between gluten and seed proteins were weak in the current samples, some of the physicochemical attributes could still be altered by seed protein addition due to the presence of nonprotein impurities in the concentrates.

The composition of the seeds investigated here are characterized by considerably high concentrations of protein and carbohydrates. In black cumin seeds, crude carbohydrate and protein content correspond to approximately 37 and 20%, respectively (Nergiz and Ötles, 1993). Black cumin water soluble polysaccharides were found to be primarily composed of galacturonic and glucuronic acids as well as neutral sugar groups, while their molecular weight corresponded roughly to 800 kDa (Trigui et al., 2018). Based on these findings, polysaccharides in BC seeds are considerably larger in molecular size compared to the BC proteins (Coşkun et al., 2019) which in turn could affect the interactions between water and protein molecules. In the grape seeds, carbohydrate concentration was considerably higher than protein content (i.e., 37% to 8.2%) (Kamel et al., 1985). However, in the case of pumpkin kernels or pumpkin seeds, protein content was considerably higher compared to carbohydrates (i.e., approx. 4 to 1) (Quanhong and CaiLi, 2005). The extent of water-soluble polysaccharides in pumpkin seeds was also considerably lower (1.1%) (Samant and Rege, 1989) which potentially limits their influence on the technical attributes of the PSPC. Consequently, the composition of protein concentrates and impurities present therein can be anticipated to be significantly different. The protein concentrates manufacture technique used here (AE-IP) is based on aqueous solubility. The fiber and ash components remaining in the cold press cakes can be transferred to the protein concentrates, which in turn influence the gluten and starch characteristics in the flour mix. Especially the high carbohydrate concentration in grape seeds makes it difficult to extract seed proteins and significantly affects technical attributes of the protein concentrates (Coşkun et al., 2020).

**Determination of Dough Rheology**

Strong flour mixtures are characterized by higher gluten quality and extensibility, enhanced bread loaf volume and height, while longer mixing durations enable homogeneous mixing of strong mixtures (Zaidel et al., 2010). Energy values also represent the resistance against the deformation and correlates well with the gas retention capacity of dough. Since the yardstick for the current treatments is the control dough, the extensograph characteristics based on energy and extension were compared to that of the control dough in all cases. Extensograph analysis of control, and BCPC, GSPC and PSPC enriched wheat dough samples were carried out as a function of dough resting time (45, 90 or 135 min) and the results were shown in Table 3. Compared to the controls, BCPC and GCPC enriched wheat flour doughs were more resistant to extension, whereas opposite results were...
obtained for PSPC samples. In most cases, the extension values of BCPC and GCPC samples were either lower than the corresponding controls or the changes were not statistically significant. Consequently, PSPC dough samples were relatively comparable to the control samples. Although the trends slightly changed over time, resting time dependence of the results were weak.

Farinograph analysis was carried out to determine the degree of softening and stability of the dough samples as influenced by protein enrichment and the results were reported on Table 4. Firstly, farinographic parameters of control bread were found to be comparable to the data in previous literature (Voicu et al., 2017). Water absorption of the dough samples was found to be statistically similar to the control samples. In addition, the degree of softening was lower in most cases compared to the control sample both at 10 and 12 minutes. Possibly the hardest and most stable bread dough samples were attained by the addition of BCPC. In most cases, farinograph and extensograph analyses indicated that the most significantly different attributes were attained by BCPC inclusion in the flour. PSPC containing flour and dough samples were mostly comparable to the controls, which was coherent with our earlier findings on gluten-free formulations (Coşkun et al., 2020).

Table 4. Farinograph parameters of dough samples prepared from protein-fortified flours. D- BCPC: Dough prepared from black cumin protein concentrate enriched wheat flour; D-GSPC: Dough prepared from grape seed protein concentrate enriched wheat flour; D-PSPC: Dough prepared from pumpkin seed protein concentrate enriched wheat flour. a: 10 minutes after the experiment started; b: 12 minutes after the maximum value was attained. The standard deviation was < 5% of the sample mean in all cases. One-way ANOVA with post hoc Tukey tests were carried out to compare groups and establish statistical significance (P<0.05). In all cases, at least three replicates were analyzed.

| Samples     | Water absorption (%) | Development time (min) | Stability time (min) | Degree of softening (FU)ᵃ | Degree of softening (FU)ᵇ |
|-------------|----------------------|------------------------|----------------------|--------------------------|--------------------------|
| Control     | 57.2                 | 1.5                    | 3                    | 60                       | 69                       |
| D-BCPC      | 57.8                 | 2                      | 7.2                  | 42                       | 58                       |
| D-GSPC      | 56.8                 | 1.7                    | 2.5                  | 50                       | 76                       |
| D-PSPC      | 57.6                 | 1.5                    | 3.4                  | 48                       | 66                       |

Table 5. Color parameters for baked bread crumb. C-BCPC: Crumb of black cumin protein concentrate enriched bread, C-GSPC: Crumb of grape seed protein concentrate enriched bread, C-PSPC: Crumb of pumpkin seed protein concentrate enriched bread. One-way ANOVA with post hoc Tukey tests were carried out to compare groups and establish statistical significance (P<0.05). In all cases, at least three replicates were analyzed.

| Samples     | Lᵃ       | aᵇ       | bᵇ       |
|-------------|----------|----------|----------|
| Control     | 55.6±0.9ᵃ | 2.6±0.5ᵇ | 24.09±0.1ᵇ |
| C-BCPC      | 3.01±1   | 2.09±0.1ᵇ | 8.95±0.1ᵇ |
| C-GSPC      | 32.01±0.9ᵃ | 8.03±0.4ᵇ | 13.65±0.5ᵃ |
| C-PSPC      | 55.75±0.2ᵉ | 2.01±0.5ᵃ | 23.45±2ᵇ |

**Analysis of Bread Characteristics**

Texture Profile Analysis

Texture profile analysis including firmness and springiness attributes of baked bread products prepared from control flour and protein enriched wheat flours were shown in Figure 1. Springiness values of all samples were comparable to that of control sample. In terms of firmness, however, BC samples were distinctly different from the other samples and controls. Once again, water holding capacity of the potentially glycosylated BCPC (Coşkun et al., 2019) and its resistance to extension in dough could have imparted a firmer structure. In addition to water holding capabilities, water soluble polysaccharides from black cumin were shown to demonstrate surface activity possibly due to the presence of proteins in the samples, although the structural attributes were not fully elucidated (Trigui et al., 2018). Previously it was also found out that BC enriched gluten-free bread had lower volume compared to the GS and PS samples, which is coherent with the current observations (Coşkun et al., 2020). In gluten-free formulations, current protein concentrates significantly increased the firmness of the crumb with the exception of PSPC samples, while further water inclusion lead to corrected firmness values (Coşkun et al., 2020).
Figure 1. Textural parameters of protein-fortified bread based on the protein concentrate utilized. BC: black cumin protein concentrate enriched bread, GS: grape seed protein concentrate enriched bread, PS: pumpkin seed protein concentrate enriched bread. A: Springiness (%), B: Firmness (g).

Due to the natural colors of black cumin and grape seed, their corresponding protein concentrates could be expected to influence the lightness and redness attributes, respectively. These findings were coherent with the expectations, while the other attributes were also significantly affected for both samples. No extra effort was made to remove the color from these concentrates, since natural color should be acceptable to the consumers. This statement will only hold true when the other quality attributes are acceptable. Consequently, while color might not be the sole defining factor, PSPC bread with comparable physical characteristics are more likely to be acceptable to consumers compared to BCPC and GSPC bread.

As indicated in the earlier sections, since the protein:carbohydrate ratio is fairly high in the pumpkin seed, the extent of carbohydrate impurities and water-soluble polysaccharides are lower in the AE-IP protein concentrates. Consequently, the chief attributes of the baked samples such as springiness and firmness were only slightly altered in the PSPC supplemented bread. Furthermore, when the interactions between the added protein and gluten and/or starch granules are relatively weak, seed protein supplementation could enhance protein content while minimally affecting the other physicochemical attributes. The attributes of the final bread samples were highly dependent on purity level and composition of protein concentrates as well as the fermentation duration.

Conclusions

Supplementation of wheat flour with black cumin, grape seed and pumpkin seed protein concentrates produced dough with satisfactory rheological properties. However, enriched bread, besides PSPC enriched bread had lower volumes, higher firmness and darker color attributes, whereas firmness had a clear bearing on the final attributes of the baked bread samples. The interactions between gluten or starch with seed proteins were relatively weak, which in turn did not significantly affect textural attributes with the exception of black cumin protein addition.

Current seed protein concentrates were conveniently utilized in both gluten-free and gluten-bearing bread formulations in order to valorize cold press technology by-product streams to generate both nutritionally and technically desirable attributes. The current protein manufacture processes were based on food grade operations and can be adapted to both artisanal and industrial bread manufacture processes leading to sustainability in the utilization of seed-based ingredients. The findings could be applicable to various by-product streams and seed ingredients that contain significant amounts of proteins.

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