



Pilot Plant Scale Manufacture of Bread Enriched with Seed Protein Concentrates

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<p><i>Research Article</i></p> <p>Received : 28/09/2020 Accepted : 17/05/2021</p> <p>Keywords: Cold press cakes Black cumin Grape seed Pumpkin seed Protein fortification</p>	<p>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-isoelectric 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.</p>

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Tohum Protein Konsantrelerince Zenginleştirmiş Pilot Tesis Ölçekli Ekmek Üretimi

MAKALE BİLGİSİ	ÖZ
<p><i>Araştırma Makalesi</i></p> <p>Geliş : 28/09/2020 Kabul : 17/05/2021</p> <p>Anahtar Kelimeler: Soğuk pres posası Çörek otu Üzüm çekirdeği Kabak çekirdeği Proteinle zenginleştirme</p>	<p>Birçok tohumun soğuk pres teknolojisi ile işlenmesi sürecinde, üretilen yağdan daha fazla miktarlarda proteince zengin posa (pres kekleri) açığa çıkmaktadır. Posanın değerlendirilmesi, proteince zenginleştirilmiş ve diğer özellikleri konvansiyonel ürünlere benzeyen gıdaların üretilmesi için uygun bir strateji sunmaktadır. Bu çalışmada çörek otu, üzüm çekirdeği ve kabak çekirdeği protein konsantreleri alkali ekstraksiyon-izoelektrik çöktürme tekniğine uygun olarak üretilmiştir. Protein konsantresi eklemenin un, hamur ve ekmek özelliklerine etkisi tekstür profili, gluten kalitesi ve renk özelliklerini de içermek üzere görsel özellikler açısından incelenmiştir. Gluten ve tohum proteinleri arasındaki etkileşimler çoğunlukla zayıfken, bazı fizikokimyasal özelliklerin değiştiği bulgulanmıştır. Hacim ve görsel özellikler açısından, kabak çekirdeği protein konsantresi ile zenginleştirilmiş ekmek, kontrol grubu ile benzer özellikler gösterirken, çörek otu veya üzüm çekirdeği protein konsantresi ile zenginleştirilmiş buğday unları, kontrollere göre daha fazla direnç gösterdikleri ve daha düşük uzayabilirlik değerlerine sahip oldukları bulunmuştur. Kontroller ve proteinle zenginleştirilmiş glutensiz veya gluten içeren ekmekler arasındaki benzerlikler ve farklılıklar tartışılmıştır.</p>

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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 Hoseneý, 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 (Droźłowska 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 Gülseren, 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 (FAOSTAT, 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 Hoseneý, 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 (Schneildorf, 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

2/2V freeze drier. The protein contents of the samples were determined according to AOAC (2003).

Bread Making

Bread preparation and analysis were carried out based on the internal procedures of the supporting company (Polen Foods, İstanbul, 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. Farinograph analysis was based on AACCI method 54.21 using Brabender Farinograph-E 810114, whereas extensograph analysis utilized AACCI method 54.10 using Brabender Extensograph-E 860702, Germany (AACCI, 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 reach 25% strain by a P/236 R (AACC 36 mm cylinder probe) for 60 s. The resulting firmness and springiness of the crumb were used as indicators of textural changes. The calculations were performed using Texture Exponent software (Stable Micro Systems, England). The analysis was performed 2 h after baking to ensure cooling of the crumb (Ziobro et al., 2013).

Color Parameters

The crumb color was determined using Hunter lab Colorflex EZ spectrophotometer (Germany). The instrument was calibrated using a white standard calibration plate and the color was expressed in CIE-Lab space as L* (whiteness/darkness), a* (redness/greenness) and b* (yellowness/blueness) (Jafari et al., 2017).

Statistical Analysis

All experimental data were analyzed by using SPSS (version 25, SPSS Inc., USA). One-way ANOVA with post hoc Tukey tests were carried out to compare groups and establish statistical significance (P<0.05) based on the differences in groups and between groups. Data were expressed as sample mean ± standard deviation. In all cases, at least three replicates were analyzed.

Results and Discussion

Analysis of Wheat Flour

In all cases, solvent based extraction was avoided in order to preserve the structural and functional characteristics of protein concentrates. The protein contents of the black cumin, grape and pumpkin seed protein concentrates prepared via an AE-IP methodology were found to be approx. 54.7%, 30.1% and 82.9%, respectively, as measured by the Kjeldahl method (AOAC, 2003). Recipes for bread making were adjusted accordingly (Table 1).

Basic characteristics of wheat flour (i.e., control sample) and seed protein enriched flour samples were investigated based on wet gluten, gluten index, sedimentation, delayed sedimentation, falling number and fungal falling number analyses. The results were summarized on Table 2. Wet gluten values of the control sample, black cumin protein concentrate (BCPC), grape seed protein concentrate (GSPC) and pumpkin seed protein concentrate (PSPC) enriched wheat flours were found to vary approx. between 25-28%. In most cases, the wet gluten values did not indicate statistically significant differences compared to the controls. The values for the control sample were comparable to the earlier findings on gluten isolated from Indian wet cultivars (Singh and Singh, 2006). Similarly, gluten index measurements indicated that the inclusion of seed proteins did not significantly affect the index value, possibly since gluten content did not change and the influence of the protein concentrate addition was relatively low. Gluten index values were also comparable with the previous findings (Kaushik et al., 2015). Since gluten content and/or quality did not significantly change, once again in most cases, sedimentation and delayed sedimentation values were statistically similar to that of the control sample.

Table 1. Recipes for bread products (B-BCPC: Bread enriched with black cumin protein concentrate; B-GSPC: Bread enriched with grape seed protein concentrate; B-PSPC: Bread enriched with pumpkin seed protein concentrate). BCPC: black cumin protein concentrate; GSPC: grape seed protein concentrate; PSPC: pumpkin seed protein concentrate).

Ingredient	Control	B-BCPC	B-GSPC	B-PSPC
Water	430.0 g	430.0 g	430.0 g	430.0 g
Ice	200.0 g	200.0 g	200.0 g	200.0 g
Wheat flour	1000.0 g	1000.0 g	1000.0 g	1000.0 g
Salt	15.0 g	15.0 g	15.0 g	15.0 g
Compressed yeast	25.0 g	25.0 g	25.0 g	25.0 g
BCPC	-	27.4 g	-	-
GSPC	-	-	49.8 g	-
PSPC	-	-	-	18.1 g

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 ^a	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 ^a	98±2.12 ^a	33±0.00 ^c	36.5±0.70 ^a	399±7.1 ^a	913±4.94 ^a
F-GSPC	27.9±0.21 ^b	96±1.41 ^a	26±0.00 ^a	32.5±0.70 ^b	389±8.5 ^a	824±5.65 ^b
F-PSPC	25.1±0.07 ^a	98±1.41 ^a	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 (cm ²)	Resistance to extension (BU)	Extension (mm)	Maximum (BU)
45 minutes	Control	70±3.2 ^a	302±5.9 ^a	134±4.2 ^a	375±10.01 ^a
	D-BCPC	72±4.1 ^a	344±6.3 ^b	128±11.3 ^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 ^a	274±10.2 ^d	149±6.5 ^d	364±15.6 ^d
90 minutes	Control	100±4.5 ^b	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 ^a	620±30.2 ^b
	D-GSPC	101±6.2 ^b	572±3.6 ^c	116±10.3 ^a	692±24.8 ^c
	D-PSPC	87±1.3 ^a	413±4.2 ^d	128±4.6 ^b	508±41.3 ^d
135 minutes	Control	86±2.6 ^a	473±7.9 ^{ab}	117±5.3 ^b	542±12.35 ^a
	D-BCPC	100±6.1 ^b	544±8.1 ^b	121±8.2 ^b	637±26.3 ^b
	D-GSPC	93±3.2 ^c	576±11.6 ^b	111±9.6 ^a	662±45.6 ^c
	D-PSPC	78±4.7 ^d	389±13.9 ^a	127±4.6 ^c	476±30.2 ^d

Falling number and fungal falling number measurements indicated that in most cases, a relatively higher α -amylase activity was observed in the protein enriched samples, possibly due to the α -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 α -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 GSPC 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	L*	a*	b*
Control	55.6±0.9 ^c	2.6±0.5 ^{ab}	24.09±0.1 ^b
C-BCPC	3.01±1 ^a	2.09±0.1 ^a	8.95±0.1 ^a
C-GSPC	32.01±0.9 ^b	8.03±0.4 ^b	13.65±0.5 ^a
C-PSPC	55.75±0.2 ^c	2.01±0.5 ^a	23.45±2 ^b

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).

Visual Characteristics

Images of baked bread products prepared from control flour, BCPC, GSPC or PSPC enriched wheat flours were shown in Figure 2 as a function of fermentation duration at constant fermentation temperature (35°C) and relative humidity (90% RH).

Morphologically, the surface score of BCPC sample was not properly visible after a 90 min fermentation process. According to the extensograph data, BCPC yielded higher resistance to dough mixing and demonstrated lower extensibility. As fermentation duration increased, the fermentation stability of the BCPC bread was found to be low and geometric deformation was clearly obvious to the naked eye. On the contrary, less resistant GSPC and the least resistant PSPC samples yielded comparable geometries as the control bread.

Color Analysis

Color analysis of baked bread products prepared from control sample, BCPC, GSPC and PSPC enriched wheat flours were shown in Table 5. For almost all color parameters, PSPC sample was similar to the control sample and was hardly distinguishable from the control sample to the naked eye (Figure 2).

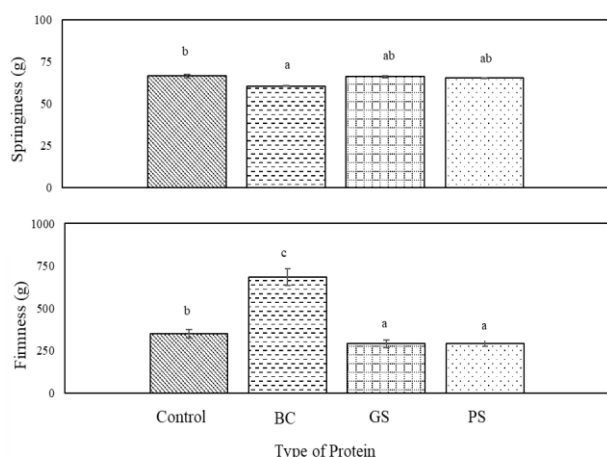


Figure 1. Textural parameters of protein-fortified bread based on the protein concentrate utilized.

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).

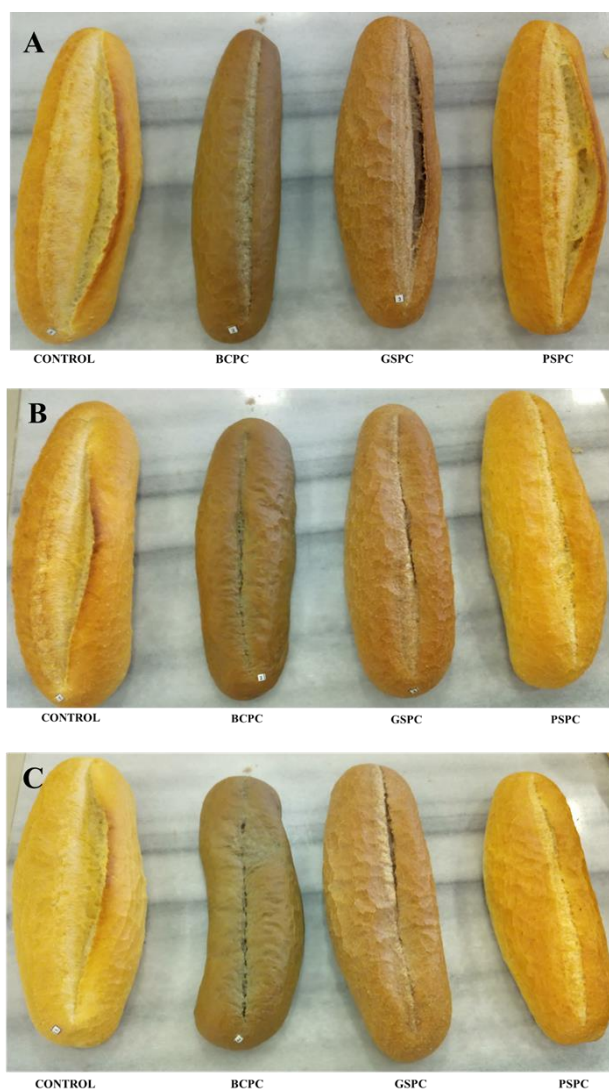


Figure 2. Morphological properties of bread with different fermentation times after baking.

Duration of fermentation; A: 80, B: 110, or C: 140 min.

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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