

## Effects of Grape Seed Powder as a Functional Ingredient on Flour Physicochemical Characteristics and Dough Rheological Properties

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### ABSTRACT

In this study, the effects of incorporating grape seed powder (GSP) on flour physicochemical properties i.e. particle size, crude protein, fiber, wet gluten and gluten quality (Zeleny test), and dough rheological properties were investigated. Increasing incorporation levels of GSP from 5 to 25% (w/w flour basis) led to an increase in the amount of fat, total phenol, and total dietary fiber; whereas total protein, wet gluten, and Zeleny values of the flour blends decreased. The results of farinograph test indicated a reduction in water absorption, stability, and farinograph quality number by increasing the concentration of GSP in the flours. Addition of GSP to wheat flour increased degree of softening and mixing tolerance of dough, while arrival time was slightly affected. Incorporation level below 10% (w/w) showed no negative effect of grape seed on dough rheological performance. There was a good potential in increasing nutritional and health value of bread by incorporating GSP, while keeping attention on the negative effects of the higher concentrations of GSP.

**Keywords:** Farinograph, Omega-3 fatty acids, Rheology, Wheat flour, Zeleny values.

### INTRODUCTION

In recent years, there is a need to apply agricultural by-products such as fruit seeds in the diets. The waste materials of grape seed pulp (seeds, stem, and peel) in grape processing industries contain lipid, protein, carbohydrate, and polyphenols. Unfortunately, these waste materials are generally utilized as cattle feed in many countries, while grape seed has been shown to contain high amounts of antioxidant compounds (Shaker, 2006; Guendez *et al.*, 2005; Burçin Özvural and Vural, 2011; Baydar *et al.*, 2004; Amico *et al.*, 2004) and is a good source of polyphenols which contain a wide variety of proanthocyanidins (Janisch *et al.*, 2006). Many consumers

increasingly demand functional food to improve their diets because they have realized the role of diet in the prevention of human diseases such as cancer, atherosclerosis, heart disease, osteoporosis, or obesity.

Bakery products have been the main constituent in the diets of most populations for thousands of years. Thus, consumption of these products supplemented with various nutritious and protective substances is advised (Mildner-Szkudlarz *et al.*, 2011). Grape seed powder (GSP) can be regarded as a functional ingredient to improve the nutritional properties of bakery products. This also helps to reduce waste disposal problems. In spite of having different health benefits, GSP has not yet attracted much attention. There have been some reports on

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enhancing the nutritive value of cereal-based products by supplementation of white bread with minerals, dietary fiber, and polyphenols (Vergara-Valencia *et al.*, 2007; Ulutas *et al.*, 2008; Mildner-Szkudlarz *et al.*, 2009, 2010, 2011; Peng *et al.*, 2010; Binzer, *et al.*, 2011; Clifford Hoye and Carolyn, 2011; Ghanbari and Farmani, 2013; Majzoobi, *et al.*, 2013). Application of GSP has the advantages of incorporating all constituents of grape waste including oil fraction with valuable fatty acids for human health, dietary fiber and phenolic compounds. Thus, there is a good potential for fortification of bakery products, especially bread as a central constituent in the diets of most populations. This study focused on the effect of incorporating GSP on flour physicochemical characteristics and dough rheological properties using farinograph test.

## MATERIALS AND METHODS

Wheat flour with an extraction rate of 80% was supplied by Athar Co. (Bonab, Iran). Black grape by-product was kindly provided by Takdaneh Co. (Marand, Iran) from the vintage of 2011 harvested in Sardasht, Kordestan province, Iran. The material was sun-dried up to a moisture content of approximately 6-8% dm. Skins were separated from seeds using a sieve with a mesh of 3-5 mm. The seeds were then cleaned from extraneous impurities and grounded in a commercial coffee grinder KENWOOD (OWCC-100002) to particle size range of less than 800  $\mu\text{m}$ . Powdered grape seed was stored at  $-18^{\circ}\text{C}$  in a freezer until subsequent analysis.

Moisture, ash, total protein, crude fat, total dietary fiber, wet gluten content, and Zeleny value of the wheat flour were determined according to AACC approved methods 44-15A, 08-01, 46-13, 30-10, 32-05.01, 38-12 and 54-11, respectively (AACC, 1990). Wheat flour was blended with GSP in concentrations of 5, 10, 15, 20, and 25% (w/w) (Nassar *et al.*, 2008). GSP and flour blend (2 g) were subjected to 20 mL

methanol in a 50 mL glass flask and shaken for 30 minutes followed by centrifugation at  $4,000\times g$  for 10 minutes. Supernatants were then collected for total phenolic content measurement. Total phenolic content of the flour blends were determined by the Folin-Ciocalteu method (Swain and Hillis, 1959). A standard curve was created using different concentrations of caffeic acid and results were reported as the milligrams of caffeic acid per gram of flour. Fe, Zn, Cu, Ca, Mn, and Se determination was performed by Inductively Coupled Plasma-Optic Emission Spectroscopy (ICP-OES). Mixing was carried out using a laboratory mixer (Behringer, Germany) until a uniform and homogenous blend was obtained. The blend was then stored in air-tight containers in a cold atmosphere until subsequent characterizations. Particle size distribution was measured by ASAE standard (Rajabzadeh, 1992) using a laboratory plansifter (Aras Mehr Co, Iran) having different meshes ranging from 150 to 475  $\mu\text{m}$ . Water absorption and dough rheological characteristics were determined by a 50 g mixing bowl of Farinograph (Brabender, model FE022N, Germany) according to AACC method 54-21.

## Statistical Analysis

All measurements were carried out in triplicate and data reported in tables and figures are mean  $\pm$  standard deviation. Analysis of variance and Duncan tests were conducted to determine significant ( $P < 0.05$ ) differences among the means using SPSS 16.0 statistical package (Berenji-Ardestani *et al.*, 2008).

## RESULTS AND DISCUSSION

### Physicochemical Characteristics of Wheat Flour and GSP

Results of chemical characterizations of wheat flour and GSP are given in Table 1.

**Table 1.** Chemical composition of GSP and wheat flour (g 100 g<sup>-1</sup> dm).

Properties	Grape seed powder	Wheat flour
Moisture (%)	7.48 ± 0.73	13.68 ± 0.10
Ash (%)	2.45 ± 0.18	0.74 ± 0.02
Total dietary fiber (%)	42.74 ± 0.6	0.04 ± 0.031
Fat content (%)	17.11 ± 0.17	5.47 ± 0.06
Crude protein Content (%)	10.94 ± 0.06	14.52 ± 0.08
Wet gluten (%)	—	27.67 ± 0.58
Zeleny sedimentation value (cm <sup>3</sup> )	—	26.3 ± 0.1
Falling number (s)	—	433.67±4.16
Total phenol (mg Caffeic acid g <sup>-1</sup> )	35.99 ± 0.62	3.6 ± 0.34

Ash content of GSP was about 2.45±0.18 g 100 g<sup>-1</sup> (dm). This was in agreement with previous reports (Kim *et al.*, 2006; Goni *et al.*, 2005; Cao and Ito, 2003). Results showed that increasing GSP led to a decrease in flour moisture content, increase in total dietary fiber, fat, and total phenol contents as shown in Table 2.

Fiber content of GSP was found to be higher than that of wheat flour. Thus, incorporating this material into wheat flour will increase the fiber content of the product. Total dietary fiber of GSP and flour blends significantly increased ( $P < 0.05$ ) from 0.04 to 9.8% depending on the amount of applied GSP. Grape seed has more than 40% dietary fiber and less than 20% oil (Kim *et al.*, 2006; Choi and Lee, 2008). The fat contents for all treatments increased significantly ( $P < 0.05$ ) upon adding the GSP. GSP had lower levels of protein compared to wheat flour control (Table 1). Statistical analysis revealed no significant ( $P < 0.05$ ) differences among 5, 10, and 15% treatments. Significant ( $P < 0.05$ ) differences were found between flour blends and the control sample in terms of phenolic compounds values which were more pronounced for 20 and 25% incorporation level. Black grape fruit and seeds are good sources of valuable phenolic and antioxidant substances. It is therefore expected that flours with higher GSP incorporation levels contain more phenolic compounds. The effect of GSP addition on Zeleny sedimentation value is shown in Table 2. Zeleny number values were decreased by increasing GSP

incorporation level. This was primarily due to diluting effect of fiber-rich GSP on gluten proteins. Secondly, low quality proteins in grape seeds could reduce swelling performance of glutenin macropolymers in Zeleny test, resulting in low Zeleny sedimentation values. Change in total protein content is associated with Zeleny values. Wet gluten data revealed no significant differences among treatments containing 10 and 15% GSP compared to the control flour. Highest wet gluten values were observed for 0 and 5% GSP incorporation. Gabriela (2009) reported that increasing the level of fiber in the bread resulted in decreasing wet gluten.

### Mineral Analysis

Several trace elements, such as iron, zinc, copper, manganese, molybdenum and calcium, are components of enzymes essential for life, and should be supplied by different diet. Fe, Zn, Cu, Ca, Mn, Se contents of GSP were 1.7%, 0.7%, 0.1%, 0.0231%, 1.6, and  $1.5 \times 10^{-7}$  %, respectively.

### Particle Size of Wheat Flour and GSP

Measurement of particle size showed that incorporating GSP to wheat flour increased coarse particles (over 475  $\mu\text{m}$ ) proportion in the flour blends. Therefore, extra care must be taken to separate coarse particles in GSP powders before usage (Table 3).

**Table 2.** Chemical composition of flour blends with different percentages of GSP (g 100 g<sup>-1</sup> dm).<sup>a</sup>

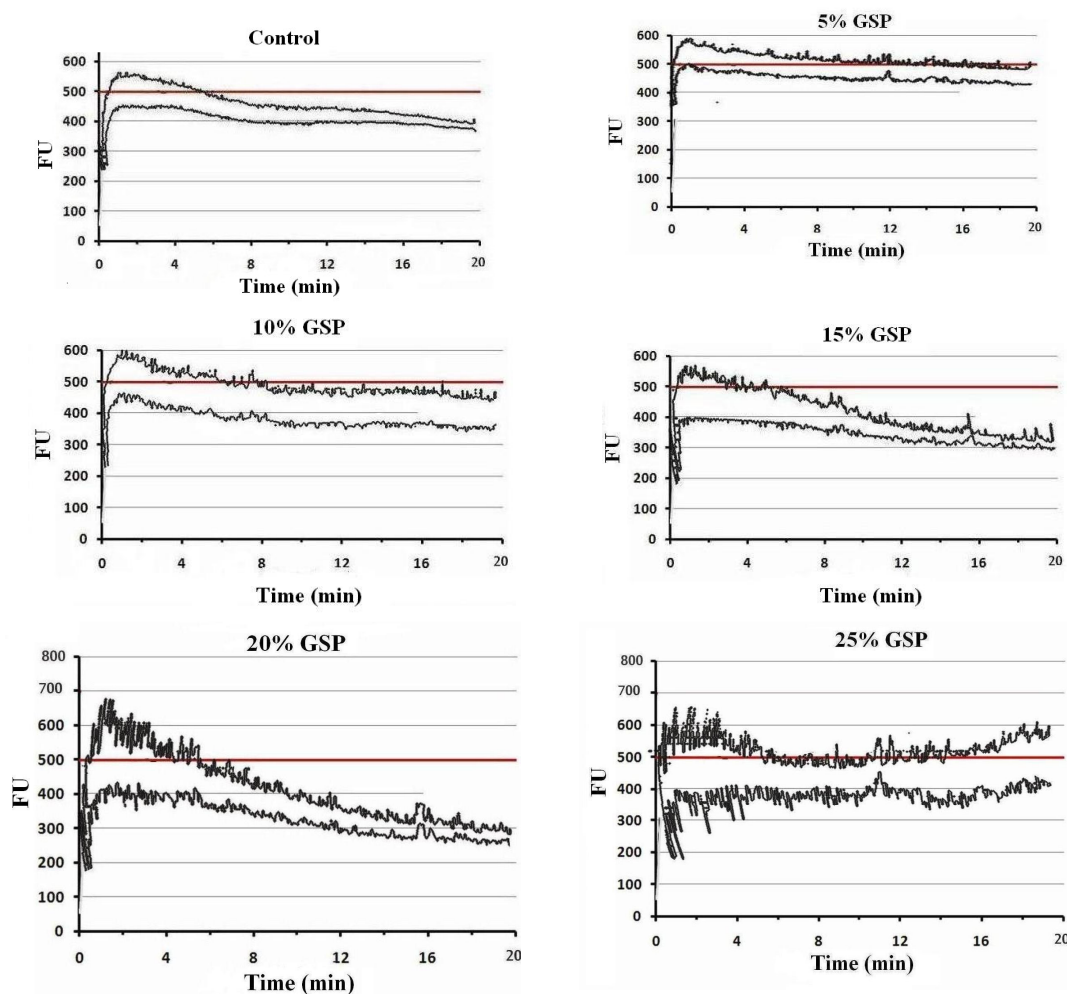
	Moisture (%)	Ash (%)	Total dietary fiber (%)	Crude protein (%)	Total fat (%)	Wet gluten (%)	Total phenol (mg Caffeic acid /g)	Zeleny sedimentation value (cm <sup>3</sup> )
Control	13.68±0.1 <sup>a</sup>	0.74±0.02 <sup>d</sup>	0.04±0.031 <sup>f</sup>	14.52±0.08 <sup>a</sup>	5.47±0.06 <sup>f</sup>	27.67±0.58 <sup>a</sup>	3.6±0.34 <sup>f</sup>	26.30±0.1 <sup>a</sup>
5% GSP	13.52±0.2 <sup>ab</sup>	0.82±0.01 <sup>cd</sup>	2.10±0.07 <sup>c</sup>	14.03±0.07 <sup>b</sup>	5.63±0.03 <sup>e</sup>	29.00±1 <sup>a</sup>	4.16±0.17 <sup>e</sup>	24.53±0.06 <sup>b</sup>
10% GSP	13.17±0.2 <sup>bc</sup>	0.88±0.02 <sup>c</sup>	4.43±0.11 <sup>d</sup>	13.65±0.17 <sup>c</sup>	6.09±0.08 <sup>d</sup>	28.00±1 <sup>a</sup>	5.22±0.06 <sup>d</sup>	22.63±0.38 <sup>c</sup>
15% GSP	12.82±0.4 <sup>cd</sup>	0.94±0.02 <sup>bc</sup>	6.58±0.24 <sup>c</sup>	13.52±0.04 <sup>cd</sup>	6.98±0.11 <sup>c</sup>	27.67±1.53 <sup>a</sup>	6.80±0.06 <sup>c</sup>	18.63±0.25 <sup>d</sup>
20% GSP	12.63±0.2 <sup>dc</sup>	1.05±0.05 <sup>ab</sup>	8.46±0.33 <sup>b</sup>	13.41±0.08 <sup>d</sup>	7.30±0.04 <sup>b</sup>	25.67±0.58 <sup>b</sup>	8.93±0.06 <sup>b</sup>	15.57±0.25 <sup>e</sup>
25% GSP	12.17±0.1 <sup>e</sup>	1.12±0.01 <sup>a</sup>	9.85±0.26 <sup>a</sup>	13.20±0.03 <sup>e</sup>	7.63±0.03 <sup>a</sup>	25.67±1.53 <sup>b</sup>	10.89±0.17 <sup>a</sup>	12.20±0.36 <sup>f</sup>

<sup>a</sup> Different letters in columns show significant (P<0.05) differences between means.

**Table 3.** Particle size distribution of flour incorporating grape seed powders.<sup>a</sup>

Treatments	Over 475 μm	Over 200 μm	Over 180 μm	Over 150 μm	Under 150 μm
Control	5.04±0.8 <sup>f</sup>	57.2±1.34 <sup>a</sup>	25.7±1.48 <sup>a</sup>	4.36±0.33 <sup>a</sup>	4.28±0.87 <sup>b</sup>
5% GSP	10.06±0.94 <sup>e</sup>	53.5±1.33 <sup>b</sup>	23.92±0.9 <sup>b</sup>	4.28±0.61 <sup>a</sup>	4.69±0.56 <sup>b</sup>
10% GSP	15.66±0.73 <sup>d</sup>	52.22±0.98 <sup>bc</sup>	20.27±1.15 <sup>c</sup>	3.48±0.33 <sup>a</sup>	5.7±0.58 <sup>ab</sup>
15% GSP	19.99±0.17 <sup>c</sup>	51.01±0.98 <sup>cd</sup>	18.73±0.69 <sup>d</sup>	3.67 ±0.25 <sup>a</sup>	5.17±0.38 <sup>ab</sup>
20% GSP	23.95±0.46 <sup>b</sup>	49.25±1.18 <sup>d</sup>	14.99±0.36 <sup>e</sup>	3.61±0.28 <sup>a</sup>	4.99±0.09 <sup>a</sup>
25% GSP	27.56±1.07 <sup>a</sup>	46.97±0.89 <sup>e</sup>	14.22±0.31 <sup>e</sup>	3.45±0.93 <sup>a</sup>	5.05±0.92 <sup>a</sup>

<sup>a</sup> Different letters in columns show significant (P<0.05) differences between means.

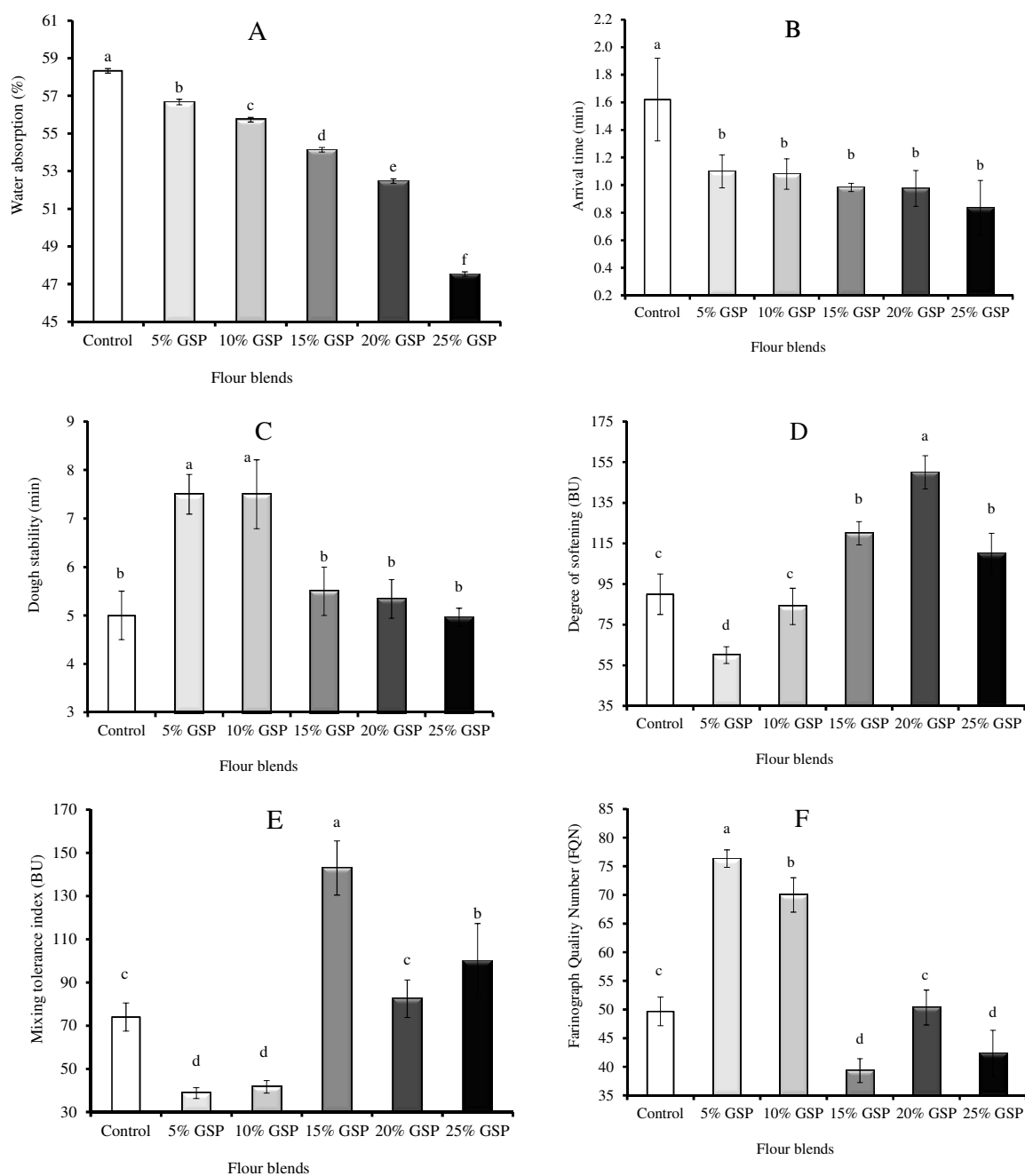


**Figure 1.** Farinograms of wheat flour (control) and different combinations of grape seed powder/ flour blends (5, 10, 15, 20 and 25%, flour basis). FU and GSP in the figures represent "Farinograph Units" and "Grape Seed Powder", respectively.

### Farinograph Results

Figure 1 shows farinograph curves derived from GSP/wheat flour treatments. Farinograph properties such as water absorption, arrival time, degree of softening, dough stability, mixing tolerance index, and Farinograph quality number (FQN) (Figure 2) were evaluated. Water absorption (WA) of the GSP containing wheat flours significantly decreased by increasing GSP content from 0 to 25% (Figure 2-A). This could be due to the increase in

concentrations of chemical compounds with hydrophobic properties, such as fatty acids. Although GSP has fiber-rich fractions, however its oil content is high and is regarded as oily additive which obviously reduced water-holding capacity of the flour by reduced farinograph water absorption (Binzer *et al.*, 2011). As shown in Figure 2-B, increasing GSP level considerably reduced arrival time of dough in farinograph. Arrival time is an indicator of hydration capacity of flour particles, and, therefore, presence of a hydrophobic



**Figure 2.** Farinograph characteristics wheat flour (control) and different combinations of grape seed powder (GSP) / flour blends (5, 10, 15, 20 and 25%, flour basis). A-F represent: water absorption (A), arrival time (B), dough stability (C), degree of softening (D), mixing tolerance index (E) and farinograph quality number (F). Error bars indicate SD values. Different letters indicate significant differences ( $P < 0.05$ ) between means.

component in GSP such as fatty material could be responsible for this observation of arrival time. Dough stability data for flours enriched with GSP is shown in Figure 2-C. Incorporation level up to 10% increased dough stability in farinograph mixing trials. This might be due to the presence of fatty compounds in GSP, which interfere with polymeric fraction of gluten leading to softening of dough and improvement of dough rheological performances. However, incorporating more than 10% did not increase dough stability anymore. Probably, higher fiber contents of GSP were responsible for reduction of dough stability at higher incorporation levels. Moreover, as observed for reduced wet gluten contents (Table 2), increasing GSP level led to weakening of the polymeric gluten content and this could be the reason for reducing gluten network formation and dough stability in farinograph. Also, this finding is in consistent with Zeleny number data, showing significant decrease in protein quality with addition of GSP. In Figure 2-D, degree of softening of the dough after 12 minutes is shown. With addition of GSP, the degree of softening increased, indicating dough weakening and reduction of tolerance of the dough. This is due to the fact that the addition of GSP diluted the glutenins in flour mixtures and thereby weakened the crosslinks between the proteins. This would lead to weakening the interactions between the chains influencing the formation and expansion of gluten network. Mixing tolerance index (MIT) data showed that increasing GSP level in flour blends increased MIT values, which led to dough weakening (Figure 2-E).

Farinograph quality number of flour blends are shown in Figure 2-F. This number describes the general mixing quality of flour in a single number, instead of calculating different multiple indices of farinograph curve. Weak and strong flours represent low and high FQNs, respectively. The highest number of FQN was obtained from sample containing 5% GSP. Reduction in FQN is due to diminishment of dough

stability and enhancement of softening degree of dough containing GSP [Figure 2, (C and D)]. Based on the published reports, the farinograph parameters of dough stability and FQN decreased, whereas the mixing tolerance index increased with increasing amounts of additive in the blends (Sudha *et al.*, 2007; Sharma *et al.*, 1999; Dervas *et al.*, 1999).

## CONCLUSIONS

Grape seed is an excellent source of dietary fiber and omega-3 fatty acids. In this study, effect of incorporating GSP on flour quality characteristics and the rheological properties was investigated. Incorporating GSP in wheat flour decreased total protein, wet gluten, and Zeleny sedimentation value, whereas an increase in total dietary fiber, fat, and total phenol was observed. Addition of GSP in wheat flour led to a decrease in the water absorption, arrival time, FQN, and dough stability in farinograph, while an increase was observed in the degree of softening and mixing tolerance. Physicochemical characteristics and farinograph results of GSP-blended dough revealed that a maximum amount of 10% GSP could be incorporated into the flour to achieve acceptable physical properties. However, including flour improvers such as emulsifiers, hydrocolloids, and oxidizing agents could allow for further enrichment of the flour with grape seed by-products.

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### اثر افزودن پودر هسته انگور به عنوان افزودنی عملگرا بر خصوصیات فیزیکی شیمیایی آرد و ویژگی‌های رئولوژیکی خمیر

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#### چکیده

در این مطالعه اثر افزودن پودر هسته انگور بر خصوصیات فیزیکی شیمیایی آرد (مانند اندازه ذرات، پروتئین خام، فیبر، گلوتن مرطوب و کیفیت گلوتن (آزمون زلنی)) و ویژگی‌های رئولوژیکی خمیر مورد مطالعه قرار گرفت. افزایش سطوح جایگزینی پودر هسته انگور از ۵ تا ۲۵ درصد، باعث افزایش میزان چربی، فنل کل و فیبر رژیمی گردید، در حالی که پروتئین کل، گلوتن مرطوب، رسوب زلنی مخلوط‌های آرد کاهش یافت. هنگامی که غلظت‌های بالای پودر هسته انگور استفاده گردید، نتایج آزمون فارینوگراف، کاهش جذب آب آرد، پایداری و عدد کیفی فارینوگراف را نشان داد. افزودن پودر هسته انگور به آرد گندم درجه نرم شدگی خمیر و قدرت تحمل مخلوط کردن را افزایش داد، در حالی که زمان رسیدن را اندکی تحت تأثیر قرار داد. نتایج نشان داد که مخلوط ۵ و ۱۰ درصد پودر هسته انگور حالت رئولوژیکی مناسب‌تری در داده‌های فارینوگراف ایجاد می‌کند. پتانسیل مناسبی در افزایش ارزش تغذیه‌ای و سلامتی محصول به وسیله افزودن پودر هسته انگور وجود دارد. به هر حال، افزودن پودر هسته انگور در مقادیر بالا باعث افت ویژگی‌های رئولوژیکی می‌گردد.