Physicochemical, Nutritional, Shelf Life and Sensory Properties of Iranian Sangak Bread Fortified with Grape Seed Powder

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Abstract

Grape seeds from grape pomace, a waste product generated during juice extraction, contain a high amount of fiber, valuable fatty acids, proteins, sugars, mineral, antioxidants and polyphenols. Grape Seed Powder (GSP) was explored for use in bread production due to its potential health benefits. Dough and breads were prepared using different levels of replacement of wheat flour with GSP (5, 10, 15 and 20%, on flour basis). Bread sensory properties during three days of storage were assessed. Unsaturated fatty acids and tocopherols were also measured before and after bread baking. Incorporating GSP in bread formulations significantly (p<0.05) influenced sensory parameters of breads. Addition of more than 10% GSP decreased overall acceptability of bread. The composition and content of fatty acids and tocopherols was not significantly (P>0.05) changed after baking. With increasing GSP concentration, color values of L* and b* in bread decreased, while a* value increased. Results showed that increasing GSP in the flour blends resulted in decreased moisture content and water activity in the final product, leading to an extended shelf life.

Keywords: Bread; Grape seed powder; Fatty acids; Quality

Introduction

Nowadays, more attention has been focused on the utilization of food industry by-products. Grape juice extraction generates waste in the form of grape pomace (GP), seeds, and skins that remain after the fruits have been pressed. Based on some estimates, approximately 20% of the grape total weight is waste in the form of seeds, stems, and skins, which translate to over 8 million tons worldwide of GP, waste [1,2]. Removal of GP is costly, and if it is not treated effectively, it poses a serious environmental problem [3]. GP is usually used as a feed for animals due to its high fiber content [4]. It is a complex matrix containing approximately 40% fiber, 16% oil, 11% proteins and 7% complex phenols, in addition to sugars, mineral and salts [5]. Oil content of grape seeds strongly depends on grape variety and fatty acids composition which is adjusted to the following values: 61-73% linoleic acid (C18:2), 14-25% oleic acid (C18:1), 7-13% palmitic acid (C16:0), 3-6% stearic acid (C18:0), 0-0.9% palmitoleic acid (C16:1), 0-0.6% linolenic acid (C18:3), 0-0.2% myristic acid (C14:0) [6].

In addition to finding an application for a waste product, the addition of GP to foods may provide additional health benefits. Grape seeds have a high content of phenolic compounds such as gallic acid, monomeric phenolic compounds (catechin and epicatechin), and dimeric, trimeric, and tetrameric proanthocyanidins [7]. Polyphenolic compounds have been associated with increased antioxidant properties and cardioprotective effects [8], anti-inflammatory effects [9], anti-ulcer activity, and anti-carcinogenic activity [10]. Grape seed oil exhibits many pharmaceutical activities, such as properties against the oxidation of low-density lipoproteins, dilatation of blood vessel, reduction of cholesterol in serum, inhabitation of cardiovascular diseases, prevention of thrombosis and regulation of autonomic nerve [11]. In addition, grape seed oil shows antioxidant and antimicrobial properties [12,13].

GP can be dried and milled to form powder (GSP). The inclusion of GSP in bakery products such as cookies, cakes, breakfast cereals, snacks or specialty breads provides a novel method to solve a waste disposal problem while adding extra health benefits. The inclusion of GSP in bread production is of particular interest due to the popularity of this product. Bread is considered as the main diet in the most countries. Diverse bread types enriched with combinations of oilseeds are being readily accepted by consumers. However, before bread can be produced containing GSP, research needs to be performed to determine appropriate wheat flour replacement values and the impact that the incorporation of GSP in the flour has on the physicochemical properties and the sensory profile of the final bread. Thus, the overall objective of this study was to evaluate physicochemical, nutritional and the sensory properties of most common Iranian flat bread; Sangak made with varying levels GSP.

Materials and Methods

Materials

Wheat flour was kindly supplied by Aras-Mehr Co. (Tabriz, Iran) with a moisture, protein, ash and falling number contents of 12%, 10%, 1% and 322 s, respectively.

Grape pomace was procured from fruit juice industry (Takdane Co., Marand, Iran). It was sun-dried to a moisture content of approximately 8%. The peel and stem separated from seeds using a sieve with a mesh of 3-5 mm. The cleaning of grape seeds was performed manually to remove damaged seeds, dust particles and other impurities such as weeds. Grape seeds were then milled with a commercial coffee grinder to particles size range of less than 300 μm. Fresh instant dry bakery yeast were purchased from Razavi Co (Mashhad, Iran). Reagents and solvents for chemical analysis were purchased from Merck and were analytical grade.

Methods

Preparation of sangak bread: ‘Sangak’ in Persian means little...
stone. The reason for this goes back to the way this bread has been traditionally baked: on a bed of hot tiny river stones in an oven. Sangak is normally produced from wholemeal wheat flour. Bread dough formulation was: 2000 g flour, GSP (in concentrations of 5, 10, 15 and 20% on flour basis), 1700 ml tap water, 20 g baker’s yeast and 20 g salt. Breadmaking was carried out according to the traditional method for Sangak bread by an expert baker. All ingredients were mixed in a spiral mixer (Sepah-Kar, Isfahan) at a speed of 40 rpm for 10 min at room temperature to obtain a homogenous dough meeting the consistency requirements of Sangak dough. Dough was subsequently fermented for 1 h at 30 ± 3°C. Dough pieces (400 g) were sheeted and slid onto the hot pebbles of the oven by a special convex pebbles. Baking was performed at 300°C for 4 min. Breads were let cool at ambient temperature before packaging. Bread pieces were packed in plastic bags and kept at 25°C before analysis. Baking trials were carried out in duplicate.

Physicochemical evaluation of the sangak bread: Moisture content was determined by AOAC Method 925.40 [14]. Water activity was measured using a water activity meter (Rotronic) [15] at 25°C. Samples were cut into squares of 2×2 cm2 and placed in the image processing system and images were taken from breads in a stable room [16]. Crust color attributes Hunter L*, a*, b* and E were recorded using the Photoshop software version 7.

Bread shelf life: Bread shelf-life was evaluated during 5 days of storage. For this reason, the packed bread slices were stored at 25°C and checked daily for the manifestation of visible mold growth. The time for the molds to become visible in the bread was considered as bread shelf-life [17].

Sensory evaluation: Sensory evaluation was performed using 15 trained panelists. Panelists were asked to evaluate each bread piece (20×20 cm slices) using a questionnaire for sensory quality (appearance, taste, softness, chewiness, flour, and overall acceptability). Bread samples were evaluated 1 h after baking and on days 1st, 2nd, and 3rd. Sensory evaluation form consisted 5 distinct points on hedonic scale (1=extremely dislike, 3=neither like nor dislike, and 5=extremely like). Panelists rinsed their mouths with tap water between each evaluation.

Evaluation of fatty acids profile, tocopherols and tocotrienols: First, grape seed oil was extracted using the method given by Mentes et al. [18]. Esterification of the fatty acids was performed using the method given by Savage and Mcneil [19]. The fatty acids were analyzed using gas chromatography (GC) (SGE, Austin, USA) fitted with a capillary column (60 m×0.25 mm id, 0.25 µm film thicknesses). Purification of tocopherols and tocotrienols from grape seed oils were carried out using method described by Fathi-Achachlouei and Azadmard-Damirchi [20] with some modifications. The tocopherol and tocotrienol were analyzed using HPLC (KNAUER, Germany), with a KNAUER 100-C8 column (250×4.6 mm, i.d., 5 µM) eluted isocratically with acetonitril/ water (97:3, v/v) at a flow rate of 1.0 ml/min. Peaks were detected by fluorescence using an excitation wavelength of 290 nm and an emission wavelength of 320 nm.

Total Phenolic Content (TPC): Folin-ciocalteu assay

The preparation of 70% ethanol extracts of bread was modified from Wang and Zhou [21]. Briefly, 1 g of lyophilized and ground bread was weighed and defatted in 30 mL of n-hexane at 70°C for 20 min in a shaking water bath (150 rpm), after which it was dried under nitrogen. Defatted sample (0.1 g) was extracted for 12 h with 10 mL of 70% ethanol (v/v) in a shaking water bath (150 rpm) at 25°C. The extract was centrifuged at 1000 g for 15 min and filtered using Whatman Nr 1 filter paper. The extracts (supernatants) were then collected for TPC measurement and sealed in plastic scintillation vials and stored at −20°C with the headspace flushed with nitrogen. TPC of the GSP, flour and breads made with different concentrations of GSP were determined by the Folin-Ciocalteu method as reported by Hoye and Ross.

Statistical analysis

All measurements were performed in triplicate and the mean values were reported. Results presented in tables and figures are mean ± standard deviations. Data were compared using analyses of variance and by the Duncan’s multiple range test with a probability of P<0.05. The results obtained from sensory evaluation of staling rate during storage were analyzed using a completely randomized design test.

Results and Discussion

Physicochemical characteristics of Sangak bread

The effect of GSP addition on moisture content of bread upon storage is shown in Figure 1. GSP incorporated breads (at all levels of addition) had lower moisture content than the control bread. High fiber content of grape seed is responsible for this. Moisture content of bread remained constant during storage days for all treatments. Figure 2 shows variation of water activity (aw) for with increasing GSP powder in bread formulations for different storage days. aw slightly decreased as GSP level increased. This is probably related to the interference of chemical compounds, such as proteins and phenols, which bind water. At day 3, all treatments showed lower aw contents. Loss of moisture in bread could account for this lower aw values.

Colorimetric parameter of top and bottom crust is shown in Table 1. With increase in the level of GSP addition, L* (darkness–lightness) and b* (redness-greenness) values decreased, while a* (yellowness-blueyness) increased. This might be related to the fact that grape seed powder had already reddish appearance. GSP breads had relatively lower E values than those of the control bread. Our results were similar with observations of Peng et al. and Hoye and Ross [22].

Bread shelf life

Result of bread shelf life measurement based on a crucial point of mold appearance is shown in Figure 3. Increasing GSP led to a delay of mold appearance (at least two days) upon storage at room temperature. Phenolic compounds such as catechin are most probably account for this observation, as reported by Hirasawa and Takada [23].

![Figure 1: Moisture content of GSP fortified breads. Data are expressed as mean. Error bars indicate SD values. Different letters indicate significant differences (p<0.05).](image-url)
Results showed that increasing GSP in the bread samples decreased the overall acceptability score especially at addition levels of 15% and 20% (Table 2). The consumer sensory evaluation study showed that control bread was preferred compared to fortified bread containing GSP. These results are in agreement with the findings of Binzer et al. [27]. Analysis of variance showed that during last storage period had a significant effect on loss of overall acceptability scores.

**Fatty acid profile**

Fatty acid profile of GSP and bread containing 20% GSP is shown in Table 2. The subjective evaluation of bread samples was carried out for sensory characteristics i.e. porosity, softness, chewiness, taste, flavor, and overall acceptability. Results showed that increasing GSP in bread decreased softness scores especially at addition levels of 15% and 20%. The mean value for 15% and 20% breads were 3.07 and 2.93 which decreased after three days storage to 2.53 and 2.07, respectively. Previous observations showed that a correlation exists between moisture content and the softness of bread [24]. Statistical analysis showed that storage period had no significant effect on loss of porosity for all bread samples.

**Sensory evaluation**

Sensory properties of breads fortified with GSP are shown in Table 2. The subjective evaluation of bread samples was carried out for sensory characteristics i.e. porosity, softness, chewiness, taste, flavor, and overall acceptability. Results showed that increasing GSP in the bread samples led to decrease of porosity scores (Table 2). These results are in agreement with the findings of Mildner-Szkudlarz et al. [24]. Statistical analysis showed that storage period had no significant effect on loss of porosity for all bread samples.

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Supplementation of wheat bread with GSP decreased chewiness scores. Chewiness score data revealed no significant differences among treatments contained 10 and 15% GSP compared to the 20% bread. Highest chewiness score observed for control and 5% bread samples. Statistical analysis showed that storage had a significantly negative effect on chewiness score of the control bread.

Flavor, color and texture are sensory attributes mostly used by the consumer to evaluate the acceptability of bread [26]. With increasing in the level of GSP in the formulation, the sensory scores for taste, flavor and texture decreased. Breads containing 5% GSP did not differ significantly (p>0.05) with the control. Statistical analysis showed that storage period had significant effect on loss of taste, flavor and texture.

**Table 1**: Colorimetric parameters of top and bottom crust of bread samples

|                | L* Top crust | a* Top crust | b* Top crust | E Top crust | L* Bottom crust | a* Bottom crust | b* Bottom crust | E Bottom crust |
|----------------|--------------|--------------|--------------|-------------|----------------|----------------|----------------|----------------|
| Control        | 40.09 ± 2.61a| -7.32 ± 1.32a| 36.78 ± 3.98a| 53.67 ± 2.51a| 39.75 ± 1.84a  | -4.47 ± 0.51a  | 35.87 ± 2.02a | 55.02 ± 2.05a |
| 5%GSP          | 30.64 ± 1.74a| -4.72 ± 1.74a| 24.30 ± 4.31a| 42.53 ± 2.53a| 31.05 ± 0.64a  | -4.39 ± 1.32a  | 28.62 ± 3.69a | 39.48 ± 4.04a |
| 10%GSP         | 29.23 ± 0.90a| -4.08 ± 0.80a| 26.29 ± 0.74a| 37.81 ± 0.44a| 28.23 ± 0.20a  | -4.59 ± 0.44a  | 24.39 ± 0.63a | 39.56 ± 0.65a |
| 15%GSP         | 21.64 ± 1.07a| -3.99 ± 0.40a| 20.44 ± 0.54a| 29.86 ± 2.39a| 20.51 ± 0.67a  | -3.58 ± 0.82a  | 21.36 ± 2.56a | 30.05 ± 0.63a |
| 20%GSP         | 21.32 ± 0.22a| -3.00 ± 0.39a| 19.22 ± 0.63a| 23.38 ± 1.32a| 16.38 ± 1.21a  | -2.50 ± 0.23a  | 16.48 ± 0.76a | 28.87 ± 0.53a |

Different letters in columns show significant (p<0.05) differences between means

**Figure 2**: Water activity (aw) of GSP fortified breads. Data are expressed as mean. Error bars indicate SD values. Different letters indicate significant differences (p<0.05).

**Figure 3**: Bread shelf life. Data are expressed as mean. Error bars indicate SD values. Different letters indicate significant differences (p<0.05).

**Figure 4**: Stability of grape seed oil during baking. Data are expressed as mean. Error bars indicate SD values. Different letters indicate significant differences (p<0.05).
that grape seed powder can be used to replace wheat flour in bread production, with moderate impact on sensory properties of the bread. Based on all results, the replacement of no more than 10 g GSP/100 g flour is recommended for GSP fortified bread, maintaining acceptable physical, nutritional and sensory properties. For more GSP addition, extra care must be taken to compensate the loss of bread sensory and functional properties.

**Total Phenolic Content (TPC)**

Huang et al. [34] evaluated different antioxidant assays, including TPC, FRAP, TEAC, and DPPH, concluding that TPC determined by the Folin-Ciocalteau assay is an effective method to quantify antioxidant capacity. The TPC in breads made with GSP significantly (p<0.05) increased with increasing concentrations of GSP (Figure 6). TPC increased by at least 3-fold as GSP replacement increased from 0 to 20 g GSP/100 g flour. These results reveal that GSP fortified bread contains relatively high concentrations of phenolic compounds which are important from nutritional point of view. Phenolic antioxidants are important class of food ingredients that can be added to introduce extra health benefits to various value-added food products.

**Conclusion**

Incorporating GSP to bread samples lead to decrease of moisture content and water activity in the final product. Addition of GSP caused a decrease in L* and b* values, while a* values increased in bread crust color. Increasing GSP in bread resulted in an extended shelf life by delaying mold appearance on the surface of breads. Sensory attributes of breads reversely affected as the level of GSP in the bread increased. Tocotrienols, tocopherols, and fatty acids composition were not significantly (p>0.05) changed after baking. This study shows that grape seed powder can be used to replace wheat flour in bread

| Porosity | Softness | Chewiness | Taste, flavor and texture | Overall acceptability |
|----------|----------|-----------|---------------------------|-----------------------|
| 0-day    | 1-day    | 3-day     | 0-day                     | 1-day                 | 3-day                     | 0-day | 1-day | 3-day | Overall acceptability |
| Control  | 4.47 ± 0.64 | 4.13 ± 0.74 | 4.13 ± 0.74 | 4.2 ± 1.08 | 4.07 ± 0.76 | 3.07 ± 1.16 | 4.67 ± 0.82 | 3.93 ± 1.3 | 3.13 ± 1.12 | 4.67 ± 0.82 | 4.07 ± 0.8 | 3.47 ± 1.08 | 4.07 ± 0.49 | 4.2 ± 0.41 | 3.47 ± 0.64 |
| 5%GSP    | 3.73 ± 1.16 | 3.72 ± 1.28 | 3.33 ± 1.35 | 4 ± 0.76 | 3.67 ± 1.11 | 2.93 ± 1.1 | 3 ± 0.76 | 3.53 ± 1.22 | 3 ± 0.76 | 3.13 ± 0.76 | 3.8 ± 0.84 | 3.4 ± 0.74 | 4.13 ± 0.39 | 3.67 ± 0.62 | 4.4 ± 0.64 |
| 10%GSP   | 3.27 ± 1.16 | 3.27 ± 0.66 | 3.53 ± 0.66 | 3.07 ± 0.36 | 2.67 ± 0.66 | 3.27 ± 1.12 | 2.93 ± 1.1 | 2.87 ± 0.66 | 3.8 ± 0.54 | 3.53 ± 1.12 | 3 ± 0.65 | 3.53 ± 0.51 | 3.33 ± 0.49 | 3 ± 0.65 |
| 15%GSP   | 3.07 ± 1.16 | 2.73 ± 1.03 | 2.67 ± 1.11 | 3 ± 0.76 | 2.53 ± 0.76 | 3.07 ± 0.03 | 2.93 ± 0.03 | 2.73 ± 0.12 | 2.2 ± 0.08 | 3 ± 0.53 | 2.73 ± 0.59 | 2.4 ± 0.63 |
| 20%GSP   | 3.4 ± 1.12 | 2.73 ± 1.16 | 2.47 ± 0.91 | 2.93 ± 1.22 | 2.47 ± 1.3 | 2.07 ± 0.96 | 2.93 ± 1.16 | 2.47 ± 1.18 | 2.07 ± 1.14 | 2.93 ± 1.16 | 2.2 ± 1.15 | 1.73 ± 1.03 | 2.03 ± 0.59 | 2.4 ± 0.63 | 1.93 ± 0.59 |

Different letters in columns show significant (p<0.05) differences between means.

**Table 2:** Means of sensory attributes of bread incorporated with different level of GSP

![Figure 4](image1)

The most predominant fatty acids were linoleic acid (60.69 g/100 g oil). Fatty acids composition was obtained as the following values: linoleic acid (C18:2)>oleic acid (C18:1)>palmitic acid (C16:0)>stearic acid (C18:0)>linolenic acid (C18:3). These results were in agreement with those of Bertrand and Özcan [28]. Previous observations showed that low level of linolenic acid is favorable in edible oils, since high amount of linolenic acid can produce an unfavorable odor and taste [29,30]. Sangak breads enriched with grape seed were baked at 30°C for 4 min. No changes were observed in the α-linolenic acid content and other fatty acids during the baking process. This might be related to increased concentrations of chemical compounds with antioxidant properties in GSP, such as phenolic compounds, tocopherols. Previous observations showed stability of fatty acids during the baking process [31,32].

**Tocopherol and tocotrienols**

Stability of tocopherols and tocotrienols in GSP during baking is shown in Figure 5. γ-tocotrienol, α-tocotrienol and α-tocopherol were the most abundant tocopherol while β-tocotrienol was not detected. These results were in agreement with the previously published data. Statistical analysis showed that baking process did not significantly affect the tocophorols and tocotrienols contents. Margherita et al. [33] reported that stability of different tocopherols and tocotrienols basically depends on two factors: the fatty acid composition of the oil, in particular polysaturated fatty acid content, and the type of tocopherol and tocotrienol homologues present.

**Total Phenolic Content (TPC)**

Huang et al. [34] evaluated different antioxidant assays, including TPC, FRAP, TEAC, and DPPH, concluding that TPC determined by the Folin-Ciocalteau assay is an effective method to quantify antioxidant capacity. The TPC in breads made with GSP significantly (p<0.05) increased with increasing concentrations of GSP (Figure 6). TPC increased by at least 3-fold as GSP replacement increased from 0 to 20 g GSP/100 g flour. These results reveal that GSP fortified bread contains relatively high concentrations of phenolic compounds which are important from nutritional point of view. Phenolic antioxidants are important class of food ingredients that can be added to introduce extra health benefits to various value-added food products.

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