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The Effect of Spinach Powder and Egg-Shell Powder on Physicochemical and Edible Qualities of Gluten-Free Cake

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ABSTRACT

Background: Replacement of the gluten is one of the challenging issues in the food industry, since producing nutritious and functionally acceptable gluten-free foods is difficult. The cake is a cereal-based product that attracts the consumers due to its various flavors, long shelf-life, and relatively low cost. Considering the competition in markets and the increased demand for functional and natural products, efforts have been made to improve nutritional and functional properties of the cakes by modifying their nutritional composition. **Methods:** In this research, the effect of spinach powder (0-30 %) and egg-shell powder (0-7%) was evaluated on physical characteristics (density, porosity, color, texture properties) and functional properties (Total phenol, DPPH, Ash, moisture content, crude fiber, Mineral content) of gluten free-cake. **Results:** The results indicated that increasing spinach powder reduced L^* value, a^* value, porosity, and hardness, while it increased the moisture content, density, ash, mineral content, total phenol, antioxidant activity, total fiber, and overall acceptance. Increase of the egg-shell powder increased moisture content, a^* value, total phenol and was decreased overall acceptance. On desirability function method, gluten-free cakes formulated with 11.78% of spinach powder and 4% of egg-shell powder had the most and desired physicochemical quality. **Conclusion:** Overall, the results indicated that spinach powder and eggshell powder could offer promising sources for improving the functional properties and acceptability of gluten-free cakes.

Keywords: *Gluten-free; Cake; Spinach; Egg-shell*

Introduction

Gastrointestinal diseases are the most important challenges of societies, especially in developing countries. One of these diseases is Celiac, which is a genetic autoimmune gastrointestinal disease caused by the disability to digest gluten (Niewinski, 2008). Patients with Celiac should not use some of the storage proteins (prolamins) such as gliadin (wheat), sekalin (rye), and hordein (barley) (Tsatsaragkou *et al.*, 2015, Turabi *et al.*, 2010). Replacement of the gluten is

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one of the challenging issues in the food industry, since it is difficult to produce gluten-free foods that are nutritious and functionally acceptable. Rice is one of the most important cereals to produce gluten-free products (Majzoobi *et al.*, 2017, Reguła and Kędzior, 2015, Sumnu *et al.*, 2010, Tsatsaragkou *et al.*, 2015). However, in comparison with the wheat-based products, rice-based products lack proteins, form a gluten viscoelastic network, have lower technological properties, and have some quality problems like low volume and unacceptable texture. So, use of polymeric materials, such as hydrocolloids that show viscoelastic properties similar to gluten, is unavoidable (Gularte *et al.*, 2012, Levent and Bilgiçli, 2011). The average diet in most of the Asian countries is mainly based on cereals. This diet has a low bioavailability of micronutrients and causes anemia with iron and vitamin A deficiency outbreak.

The green leafy vegetables are rich in beta-carotene (precursor of vitamin A) (Derrien *et al.*, 2017, Erfani *et al.*, 2006). Spinach (*Spinacea oleracea*) is a leafy vegetable, consumed in large quantities in some countries. Spinach production has been more than 20.79 million tons in the world since 2011 and China has had the highest production rate with 18.78 million tons (FAO, 2011). Spinach leaves are mainly consumed for their special green color as well as nutrients such as carotenes, vitamin C, and minerals like calcium and iron. Bioavailability of the minerals such as calcium and iron in leafy vegetables was measured and found to be more than 25 % (Bhople *et al.*, 2017, Bohn *et al.*, 2004, Ozkan *et al.*, 2007).

Several tons of eggshell wastes are produced every day in many countries around the word that causes lots of environmental problems (Aminah and Meikawati, 2017). The eggshell makes 9-12 % of the total egg weight and contains a large amount of calcium carbonate (94 %) and trace amounts of other micronutrients like manganese, copper, iron, magnesium, sulfur, and zinc (Shuhadah and Supri, 2009). A calcium intake from dairy products is one of the most suitable ways to resolve calcium needs. Consumption of supplements in the pill form is costly and sometimes difficult to follow. Eggshell is a calcium source, which is available at home and can be used as a calcium supplement (Krithiga and Sastry, 2011, Schaafsma *et al.*, 2000).

The cake is a cereal-based product that attracts the consumers due to its various flavors, long shelf-life, and relatively low cost (Majzoobi *et al.*, 2017, Oliveira *et al.*, 2016). Considering the competition in markets and increased demand for the functional and natural products, efforts have been made to improve the nutritional and functional properties of the cakes by modifying their nutritional composition (Levent and Bilgiçli, 2011, Lu *et al.*, 2010). Since no research has ever been conducted using the spinach powder and eggshell powder in making gluten-free cake, this study aimed to evaluate the effect of adding spinach powder (0-30 %) and eggshell powder (0-7 %) on physicochemical and sensory properties of the gluten-free rice-based cakes.

Materials and Methods

Raw materials: Rice powder (the North powdered), sugar, egg, liquid oil (Ladan Company, Iran), baking powder, vanilla, and Spinach were purchased from the local markets. To produce the required spinach powder in the cake formulation, the spinach leaves were washed, cut, and blanched for 10 minutes (the water was boiled and the spinach leaves were placed in it). After taking its water completely, the spinach was dried in the freezer dryer. The process of producing egg skin powder was so that the egg skins were supplied from the confectionery workshops and kept in the refrigerator for 12 hours. The egg skins were boiled for 10 minutes to sterilize, ground in a coffee grinder, and turned into very fine powder.

The method of preparing the cake: The cake batter was prepared according a previous study (Tsatsaragkou *et al.*, 2015). Briefly, the sugar and oil were mixed, eggs and water were added, and the powdered materials were added and stirred. The cakes were poured into the separate muffin molds immediately and placed in the pre-heated oven at 180 °C for 25 minutes. After baking, the cakes were cooled in the room temperature for 20

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min. Later, the samples were packed in the polyethylene packages with thermal sealing and kept in the room temperature until the next steps (Tsatsaragkou *et al.*, 2015). The experiments were repeated three times. The variables' levels are presented in **Table 1**.

Chemical experiments: The flour and cake moisture was measured by the approved method of AACC 44-16. The flour and samples' protein levels were measured by AACC 46-12. The flour and cake pH was measured by AOAC 02-52. The flour and cake ashes were measured by AACC08-01. Finally, the fiber of cake samples was measured using the approved method of AOAC 199-43 (Bilgiçli *et al.*, 2007).

Determination of minerals content: The minerals of cake samples and flour, such as Iron, copper, zinc, manganese, and calcium were measured using the Atomic Absorption Spectrometers (Analytikjena, Contr AA300, Germany). Measurement of calcium amount was done by titration method (Ooi *et al.*, 2012).

Determination of texture: TA-XT-PLUS (micro stable system, made in England) with a 36 mm diameter cylindrical probe, 40 % compressing, and a test speed of 0.25 mm s^{-1} was used to evaluate the hardness of samples according to AACC70-09 (Majzoobi *et al.*, 2017).

Determination of color: The color of cakes was determined using the Konica Minolta colorimeter device (CR-400 model, Japan). In this experiment, the L* value was determined as the criterion of brightness, a* value as the criterion of redness, and b* value as the criterion of yellowness.

Determination of volume: The method of volume replacement with rapeseed was used to measure the special volume, according to the AOAC10-50 standard (Eriksson *et al.*, 2014).

Determination of Porosity: In this research, the porosity amount of cake was measured and studied using an image analysis system consisting of a digital imaging camera and a personal computer. Images were placed at a fixed distance of 30 cm from samples in a black box (with approximate dimensions of 100 * 100*100 cm). The images were taken with 45-degree of lighting provided by fluorescent lamps. Later, the evaluation was conducted through an image processing method using the Image J software (version 14.2) (Nouri *et al.*, 2017).

Determination of Phenolic compounds: The sample was defatted with proportion of 1:1 V/V chloroform /petroleum ether. Next, it was dried in an oven with 40 °C. Later, 1 g of the defatted sample was mixed with 10 ml of water or methanol. The mixture was then centrifuged in Centrifuge with 2000 g for 15 minutes and 70 µL of the obtained liquid was mixed with 900 µL of distilled water, 1 ml of Folin-Ciocalteu, and 2 ml of 10% carbonate sodium. After one hour, its absorption was read in 760 nm wavelength (Sudha *et al.*, 2007).

Determination of the antioxidant properties (DDPH method): The sample was defatted with a proportion of 1:1 V/V chloroform /petroleum ether. Later, it was dried in an oven with 40 °C. In the next step, 1 g of the defatted sample was mixed with 10 ml of water or methanol, which was then centrifuged in centrifuge with 2000 g for 15 minutes. The obtained liquid was used to perform the experiment. Later, 0.1 microliter of this extract was mixed with 3.9 microliter of methanol DPPH solution. After 30 minutes of being in an incubator, it was placed in the room temperature and the samples' light absorbance was read in the wavelength of 517 nm against the blank and the percentage of DPPH free radicals inhibition was calculated with the following formula (Brand-Williams *et al.*, 1995):

I%= (A blank- A sample /A blank) *100

Sensory evaluation: The cakes were evaluated for their organoleptic characteristics (overall acceptability) by performing a five-point hedonic test using trained panelists. The panelists were asked to evaluate the samples and score them from 1 (most disliked) to 5 (most liked).

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Experiment design and statistical analysis: In this research, the central composite design was used to study the influence of independent variables on the qualitative properties of the cake. The obtained results of this design were modeled using the 7.1.6 Design.

Results

Moisture content: Variance analysis of the results showed that the fitted quadratic model was significant ($P < 0.05$) for moisture content and lack of fit was non-significant ($P > 0.05$). Moreover, results showed that the linear effect of spinach powder and eggshell powder addition was significant ($P < 0.05$) and the effect of spinach addition was higher than the eggshell. The quadratic effect of spinach powder addition was also significant ($P < 0.05$, **Table 2**).

pH: The results of variance analysis showed that the fitted quadratic model for pH was significant ($P < 0.05$) and lack of fit for this model was non-significant ($P > 0.05$, **Table 2**). As illustrated in **Table 1**, the results showed that the linear effect of spinach powder and eggshell powder addition was significant ($P < 0.05$), while the quadratic effect and interaction between variables were non-significant ($P > 0.05$, **Table 2**).

Color: According to the variance analysis results, the fitted quadratic model for L^* factor was significant ($P < 0.05$) and lack of fit for this parameter was non-significant ($P > 0.05$). As shown in **Table 2**, the linear effect of both variables was significant ($P < 0.05$), but spinach powder addition effect was greater. For a^* factor, the linear effect of both variables, the quadratic effect of spinach addition, and the interaction between both variables were significant ($P < 0.05$, **Table 2**). For b^* factor, the linear effect of spinach powder and eggshell powder addition as well as the quadratic effect of the spinach powder addition were significant ($P < 0.05$, **Table 2**).

*Density***:** The results of variance analysis showed that the fitted quadratic model for density was significant ($P < 0.05$) and lack of fit was non-significant ($P > 0.05$). According to the variance analysis results, the linear effect of spinach powder and eggshell powder addition as well as the quadratic effect of spinach powder addition were significant ($P < 0.05$, **Table 2**).

Porosity: The variance analysis showed that the fitted quadratic equation for porosity was significant ($P < 0.05$) and lack of fit was non-significant ($P > 0.05$). The linear effect of spinach powder addition was also significant ($P < 0.05$, **Table 2**).

Texture: The results of variance analysis showed that the fitted quadratic equation for this factor was significant ($P < 0.05$) and lack of fit was non-significant ($P > 0.05$, **Table 3**). According to the variance analysis, the linear effect of both variables (addition of spinach powder and eggshell powder) was significant ($P < 0.05$) and the effect of spinach powder was greater**.** By increasing the amount of spinach powder texture hardness of samples decreased significantly.

Ash and mineral content: Based on the variance analysis results, the linear effect of spinach powder and eggshell powder addition was significant ($P < 0.05$) and lack of fit for ash and mineral content was non-significant ($P > 0.05$, **Table 3**). In addition, the maximum ash content was observed in the sample with the highest spinach powder and eggshell powder percentage in the cake formulation (samples with 30% spinach powder and 7% eggshell powder). According to the variance analysis results for Iron determination, the linear effect of both variables was significant ($P < 0.05$), but the effect of spinach addition was greater. Zinc determination results showed that the linear effect of eggshell powder addition, the quadratic effect of spinach powder addition, and the interaction between these factors were significant ($P < 0.05$). According to the variance analysis in Table 3 for Calcium determination, the linear effect of eggshell powder addition and interaction between both variables were significant ($P < 0.05$). Furthermore, the linear effect of both variables, the quadratic effect of eggshell powder addition, and the interaction between eggshell powder and spinach powder addition on

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Phosphorus were significant ($P < 0.05$), while lack of fit was non-significant for all factors ($P > 0.05$).

Phenolic contents: The results of the variance analysis showed that the fitted quadratic equation for phenolic compounds was significant ($P < 0.05$) and lack of fit was non-significant ($P > 0.05$). Based on the variance analysis results, the linear effect of both spinach powder and eggshell powder addition was significant ($P < 0.05$), whereas, their quadratic effect and interaction between them were non-significant ($P > 0.05$, **Table 3**).

Antioxidant activity (DPPH): Obtained results from the variance analysis showed that the fitted quadratic equation for antioxidant activity was significant ($P < 0.05$) and lack of fit was non-significant ($P > 0.05$). The linear effect of both spinach powder and eggshell powder addition was significant ($P < 0.05$, **Table 3**).

Total fiber content: The results of variance analysis showed that the fitted quadratic equation for porosity was significant ($P < 0.05$) and lack of fit was non-significant ($P > 0.05$). The linear and quadratic effects of the spinach powder addition were significant ($P < 0.05$, **Table 3**).

Sensory evaluation: The results of the sensory evaluation variance analysis showed that the fitted quadratic model was significant for sensory evaluation ($P < 0.05$), while lack of fit was non-significant ($P > 0.05$). The linear effect of both spinach powder and eggshell powder addition was significant ($P < 0.05$, **Table 3**).

Table 1. The variables levels

Source	Moisture	pH	Color(L)	Color(a)	Color(b)	porosity	Density
Model (P-value)	0.0001^{e}	0.015^{b}	0.0001^{e}	0.0001^{e}	0.0001^{e}	0.0010^{c}	0.0001^{e}
A	10.77^{b}	15.54^{c}	6.39^{b}	55.12^{e}	6.79^{b}	3.43^{a}	9.39^{b}
B	125.25^{e}	10.84^{b}	700.29^{e}	279.20^{e}	356.55^{e}	74.76^{e}	34.15^{d}
A2	4.09^{a}	1.10^{a}	0.23^{a}	0.31^{a}	1.85^{a}	0.41^{a}	2.51^{a}
B2	5.92^{a}	5.12^{a}	213.08^{e}	220.35^{e}	81.71^{e}	0.020^{a}	91.94^{a}
AB	2.48^{a}	0.16^{a}	1.21^{a}	19.68^{c}	1.13^{a}	2.03^{a}	8.320^{e}
Lack of Fit	0.52^{a}	5.34^{a}	0.51^{a}	0.36^{a}	1.73^{a}	0.38^{a}	0.17^{a}
R-Squared	0.95	0.81	0.99	0.98	0.98	0.92	0.95
Adj R-Squared	0.92	0.68	0.98	0.98	0.97	0.86	0.93
C.V.	3.09	1.33	3.73	4.66	5.79	5.62	8.96

a: Is not significant at P < 0.05; b: Significant at P < 0.05; c: Significant at P < 0.01; d: Significant at P < 0.001; e: Significant at P < 0.0001

Table 2. Regression models for the response variables, Moisture content, pH, color, Density, Porosity.

Source	Hardness	phenolic compounds	sensory	DPPH	fiber	Ash
Model (P-value)	0.0003^{d}	0.0002^{d}	0.0073^{c}	0.0001^{e}	0.0003^{d}	0.0001^{e}
A	12.89^{c}	5.81^{b}	11.74^{b}	34.60^{d}	4.77^{a}	241.43^{e}
B	90.58^{e}	134.93^{e}	24.83^{c}	510.53^{e}	92.98^{e}	10.53^{b}
A2	2.82^{a}	0.52^{a}	0.57^{a}	0.82^{a}	0.019^{a}	1.49^{a}
B2	2.64^{a}	2.22^{a}	1.32^{a}	31.6^{d}	12.95^{c}	1.99^{a}
AB	2.22^{a}	3.03^{a}	3.46^{a}	4.75^{a}	5.55^{a}	2.70^{a}
Lack of Fit	1.88^{ns}	0.46^{a}	1.21^{a}	5.85^{a}	1.40^{a}	4.64^{a}
R-Squared	0.94	0.95	0.85	0.98	0.94	0.97
Adj R-Squared	0.90	0.92	0.75	0.98	0.90	0.95
C.V.	4.14	6.63	6.95	3.40	8.81	6.58

a: Is not significant at P < 0.05; b: Significant at P < 0.05; c: Significant at P < 0.01; d: Significant at P < 0.001; e: Significant at P < 0.0001

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Table 3. Regression models for the response variables: hardness, phenolic compounds, sensory, DPPH, fiber, and ash

Variables	Level (α)		
	-1	0	+1
Spinach powder	0	15	30
Egg shell powder	0	3.5	7

Discussion

Moisture content: Based on the results, by adding spinach powder and eggshell powder, the moisture content increased, which can be due to higher moisture content and fiber of spinach. Addition of fiber increases water absorption and water holding capacity of the cakes, which delays the samples' retro-gradation (Aydogdu *et al.*, 2018). These results are in agreement with the obtained results from similar studies (Dadkhah *et al.*, 2017).

pH: According to the results, pH decreased by increasing the spinach powder and eggshell powder. It can be due to the Hydrogen ions mobility as well as the spinach powder semi-acidity (Bhople *et al.*, 2017, Lee *et al.*, 2002).

Color: In fact, by adding spinach powder, the L^* factor decreased and the color oriented toward green, which is due to the presence of chlorophyll in spinach that reduces the cake's L^* factor (Lee *et al.*, 2002, López-Nicolás *et al.*, 2014). By adding spinach powder, a^* factor (redness) decreased, while eggshell powder addition increased this parameter.

Density: Based on the results, by adding both spinach powder and eggshell powder, the cake density increased. This density increase was greater in spinach powder addition. The maximum density observed in samples was by 30% spinach powder and 7% eggshell powder. The samples' density increase can be due to the dough viscosity reduction by adding spinach powder, which consequently decreased the ability to trap and hold the air bubbles (Majzoobi *et al.*, 2017, Oliveira *et al.*, 2016).

Porosity: Porosity is an important physical property that defines the texture and quality of dry and semi-dry foods. By increasing the spinach powder from 0 to 30%, the porosity decreased. The porosity decrease is due to the increased gas cell size, which decreased their number and uniform distribution in the product's matrix (Nouri *et al.*, 2017).

Texture: Based on the results, increasing the amount of spinach powder decreased the texture hardness and needed energy for punching the cake samples compared to the control samples. This can be due to the moisture increase by adding the spinach powder (Majzoobi *et al.*, 2017).

Ash and minerals content: The eggshell powder used in this test contained 9.02% of ash and the spinach powder had 2.7% of ash. Iranian spinach samples contain considerable amounts of important minerals such as Potassium, Magnesium, Phosphorus, Iron, Zinc, as well as fiber (Erfani *et al.*, 2006). The eggshell powder contains the highest amount of minerals (90.2%) (Aminah and Meikawati, 2017). The atomic absorption results showed that the minerals contained in samples with spinach powder and the eggshell powder were more than the control samples. In fact, by increasing the spinach powder level from 0 to 30%, the samples' Iron content increased which was due to the Iron presence in spinach (Erfani *et al.*, 2006). By increasing the eggshell powder, Zinc content increased, which is due to the high amounts of Zinc present in eggshell powder (Schaafsma *et al.*, 2000). Eggshell powder addition increased the samples' Calcium content. Schaafsma et al. found that 385-401 mg of Calcium exist in 1 gr of eggshell (depending on the eggshell itself that can be used as a Calcium source in the human diet) (Schaafsma *et al.*, 2000). Manganese, Potassium, Sodium, and Magnesium were less prevalent in eggshells. The eggshell consists of 97% solids (98% Calcium carbonate), which is about 6.4% of

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protein and a small amount of fat (Aminah and Meikawati, 2017, Brun *et al.*, 2013). So, it can be used as a Calcium carbonate source. Eggs are widely used in food industries, but eggshell is considered as a waste; therefore, access to eggshell is an important challenge for industries.

Phenolic contents: According to our results, by increasing the spinach powder and eggshell powder, the cake phenolic compounds increased; the effect of spinach powder was more significant due to the presence of phenolic compounds in it (Lee *et al.*, 2002).

Antioxidant activity (DPPH): Based on the results, by increasing the level of spinach powder, the antioxidant activity of samples increased significantly compared to the control samples, which is due to high antioxidant activity of the spinach powder (Gupta and Prakash, 2009, Lee *et al.*, 2002, Shuhadah and Supri, 2009).

Total fiber content: By increasing the spinach powder level, fiber content increased in the cake samples; so that the highest fiber content was observed in samples with 30% of spinach powder. This can be due to the spinach's high fiber content (Erfani *et al.*, 2006).

Sensory evaluation: Based on the results, by increasing the eggshell powder, the product's overall acceptability reduced. However, by increasing the spinach powder level, the sample's overall acceptance increased. Green enriched cakes with a little spinach flavor (using high levels of spinach powder) had the highest acceptance among the consumers and the maximum overall acceptance score was obtained for samples with 30% and 15% of spinach powder, respectively.

Optimization: In this research, the aim of optimization was to maximize the physicochemical properties of the fortified sponge cake with spinach powder and eggshell powder. The obtained results from optimization of the sponge cake formulation showed that the optimum amount of the factors was obtained by the following: 11.78% of spinach powder, 4% of the eggshell powder, the moisture content of 42.72%, the density index of 1.11, the color factors of a* value -7.39, b* value 18.72, and L* value 42.86, porosity of 11.06%, total phenolic content of 0.115 mg/g, antioxidant activity of 54.26%, hardness of 15.49 (N), Ash content of 3.93%, Phosphorus of 115.441 mg/Kg dry weight, Iron content of 0.274 mg/Kg dry weight, Zinc content of 0.280 mg/Kg dry weight, calcium content of 1464.9 mg/Kg dry weight, and total fiber of 3.141%.

Conclusion

After a thorough evaluation of all charactersitics of the physicochemical and functional properties, it was found that adding spinich powder had a strong effect on quality of the gluten free-cake. Overall, the results indicated that spinach powder and eggshell powder could offer a new frontier for improving the organoleptic properties and quality of gluten-free cakes. Application of the spinach powder and eggshell powder in production of the gluten-free cakes can also answer the request for a functional and natural product for people with celiac disease.

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Conflict of interest

The authors declare no conflict of interest.

Author's contributions

Asghari-pour S. and Ghasemi P. prepared the samples and carried out the experiments. Authors read and approved the final paper.

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