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Effects of Different Germinated seeds Flour on Mineral, Phytic Acid and Total Phenolic Content of Cookies

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ABSTRACT

The effects of germination at different germination period (1, 3 and 5 days) on physical, chemical and nutritional properties of three different seeds (wheat, rye and lentil) were investigated. Germination caused significant increase in ash, protein, total phenolic content, minerals (Ca, Mg, Fe, Zn, K, P) and phytic acid loss. As the germination period increased, L* values decreased, a*, b* and SI values increased. Germinated seed flours (GSF) were

substituted for wheat flour at different ratios (0, 5, 10 and 15%) in cookie formulation to improve nutritional properties. The addition of GSF in cookie formulation gave lower phytic acid content than the control cookie sample. The highest calcium and magnesium content of the cookies were determined with germinated rye flour (GRF); the highest iron, potassium and zinc value were obtained with germinated green lentil flour (GGLF). The use of 5 % level had caused similar taste values to the control cookie samples.

Keywords: Germinating; Mineral; Phytic acid; Total phenolic content

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1. Introduction

Cereals that are used in products such as bread, pasta and cookies and had a very important place in human nutrition due to the energy are the edible constituent of the grain. Legumes can be used effectively to meet the protein deficiency required for healthy and balanced nutrition in the world and in our country they are our important plant sources. Various processes (fermentation, germination, autoclaving, milling, heat application) are applied in order to ensure more efficient use of this important plant sources thus nutritional content is enriched and antimicrobial factors are reduced. Nowadays, the production and consumption of the germinated products in the world are increasing in both variety and quantity, as a result of the tendencies towards functional foods. One of the main reasons for this is that the germination process is not expensive and does not require complicated equipment (Lorenz 1980). In literature, some of the nutritionally important components such as vitamins, minerals, dietary fiber, flavonoids, phenolic acids and many antioxidant components, omega 3 type of fatty acids of plant seeds and cereals sprouts increases during germination, and also increase the functional properties of the products are stated (Siro et al 2008; Öztürk 2008). Wheat sprouts compared to wheat; in addition to higher vitamin content, it is stated that it has higher phenolic substance, higher quality protein, more aromatic amino acids and more polyunsaturated fatty acids (Öztürk 2008; Yang 2000). The phytase activity increases (Reddy et al 1982) and the phytic acid content decreases and the digestibility of the fibers and proteins changes during germination. Furthermore, the increase in amino acids and ascorbic acid contributes to the bioavailability of trace minerals (Lintschinger et al 1997). During germination, beta carotene, vitamin C and E content is increasing (Yang 2000).

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In studies, rye (Katina et al 2007), lentils (Urbano et al 1995), beans, peas (López-Amorós & Estrella 2006), cowpea, chickpeas and mung beans (Ghavidel & Prakash 2007), oats (Wilhelmson et al 2001), paddy (Kim et al 2012), lupine (Cunha-Queda & Beirao da Costa 1994), barley (Sung et al 2005), brown rice (Moongngarm & Saetung 2010) germinated.

Nutritional properties increased with germination process and germinated seeds are stated to be valuable resources of natural bioactive compounds and antioxidants. The amount of anti-nutrients decreased during the germination and after the germination, compounds with beneficial and phytochemical properties were formed. These substances are particularly important in many diseases prevention such as cancer. Thus, functional foods that protection of health and positively affected on human health can be developed by germination.

In this study, cereals and legumes (wheat, rye and green lentils) were germinated at different germination period (1, 3 and 5 days), dried and milled. Flours of cereal and legumes seeds were replaced by wheat flour at different ratios (0, 5, 10 and 15%) and cookie production was carried out to improve nutritional and functional properties.

2. Material and Methods

2.1. Material

To germinate the seeds; wheat, rye and green lentil were purchased from local market in Konya, Turkey. Wheat flour, shortening, powdered sugar, salt vanilla and sodium bicarbonate were purchased from local markets in Konya for cookie production.

2.2. Germination of seeds

Wheat, rye and lentil seeds were cleaned from all foreign material, broken and diseased grains. Seeds were germinated according to the jar method. Briefly, 100 g of cleaned seeds were placed in 500 mL of 0.07% sodium hypochlorite (NaClO) for 30 min to sanitize and then washed ten times in tap water to neutralize pH and filtered with excess water. Then, the seeds were soaked in 1000 mL distilled water for 5 hours and shaken for 30 min so as to draw water. The jars were covered with a thin cheese cloth so that the seeds were contacted with air. Then water (kernels: water ratio of 1:2) was added. After rinsing several times, the water was removed from the jar. The seeds were sprouted in the dark at room temperature with watering every 12 h for 1, 3, 5 days. At the end of 1, 3, 5 days germinated seeds were dried in 50 °C (Nüve FN-400) and grounded in a hammer mill. Ungerminated seeds were used as a control (0 day). The germinated seed flours (GSF) and wheat flour were placed in a large polyethylene bag and mixed thoroughly by hand.

2.3. Production of cookies

To prepare cookie samples; AACCI (Approved Method No: 10-54.01) method were used with some modifications. The dough was prepared in a laboratory mixer following a standard formulation by addition level of 0, 5, 10, and 15% GSF (5 day germinated wheat, rye and green lentil flour). As a result of the preliminary experiments, it was decided that the 5-day germination period was the most suitable GSF for the use of cookie production. 100 g wheat flour (according to 14% moisture content), 40 g shortening, 40 g powdered sugar, 2 g sodium metabisulphite, 1.25 g salt, 1 g milk powder 0.5 g vanillin and variable water were mixed for 10 min at 125 rpm. Cookie dough were sheeted to 5 mm thickness with a rolling pin and shaped with a dough cutter (50-mm diameter) and baked on aluminum trays in an oven (Profilo, HG1503T) at 150 °C for 16 min. The cookies were cooled for 30 min at room temperature and immediately used for further analyses.

Cookies were evaluated for physical characteristics, including diameter (mm) and thickness (mm) measured with a digital micrometer (0.001 mm, Mitutoyo, Minoto-Ku, Tokyo, Japan). Spread ratio was calculated as diameter divided by thickness of the cookies.

The cookie hardness and fracturability were investigated using the standard method AACC 74-09.01 by texture analyzer (TA-XT plus, Stable Microsystems, England). Hardness (as fracture force) of cookies with 3-point bending test using 3-point bending rig, trigger force of 50 g, and load cell of 5 kg. (Pretest speed: 1.0 mm s⁻¹, test speed: 3.0 mm s⁻¹, posttest speed: 10.0 mm s⁻¹, distance: 5 mm).

2.4. Color measurements

Konica Minolta Chroma Meter (Model CR-400, Konica Minolta Sensing, Inc., Osaka, Japan) was used for color measurement of cookies. The surface color of cookie was determined as average L* (lightness), a* (redness), b* (yellowness) values. a* and b* values were used to calculate the saturation index (SI) ($SI = \sqrt{a^{*2} + b^{*2}}$) and hue angle ($H = \tan^{-1}(b^*/a^*)$) values.

2.5. Proximal composition

Chemical composition of ungerminated seeds, GSF and cookie samples were determined following AACCI method (AACC 2000), for moisture (44-12), ash (08-01.01) and protein (46-12.01).

2.6. Nutritional analysis

Mineral content (potassium, magnesium, calcium, phosphorus, iron, and zinc) was determined in cookies using a wet digestion with closed system according to Skujins (1998) and results were expressed in mg 100 g⁻¹ sample. Phytic acid content of cookie samples was analyzed according to Haugh & Lantzsch (1983) using colorimetric method. Total phenolic content of the cookies were determined based on Folin-Ciocalteu colorimetric method as described by Gao et al (2002) and Beta et al (2005).

2.7. Sensorial evaluation

Cookies were submitted to sensory analysis by twenty (20) semi-trained panelists (50% male, 50% female) that familiar with the quality aspects of baked products. Panelists evaluated typical attributes for cookie such as color, appearance, friability, taste, odor and general appreciation using a 5-point hedonic scale.

2.8. Statistical analysis

The significance of the variations observed among cookie samples was tested according to three way analysis and one way analysis of variance (ANOVA) using software program (JUMP, version 5.0).

3. Results and Discussion

The characteristics of GSF at 3 different germination periods were shown in Table 1. A significant ($P < 0.01$) difference in L*, a*, b* and SI values were obtained according to germinated seeds and germination period as a variance factors. Lightness values ranged from 79.31 for GRF to 82.73 for germinated wheat flour (GWF). According to germinated seeds variance, while GWF showed the highest lightness value, germinated rye flour (GRF) and germinated green lentil flour (GGLF) had the highest redness and yellowness, respectively. GGLF also showed the highest saturation index and hue angle vales among the GSF. After 5 day germination period, lightness values decreased, while the redness, yellowness and saturation index values increased. As expected, decrease in lightness, probably due to the Maillard reaction (between free amino acids and sugar content) during drying. The increase of the saturation index value could be due to the increase of reducing sugar during germination. Similarly Öztürk (2008) reported a decrease in L* values and increase in a* and b* values after germination. Likewise, Ertaş (2015) and Shin et al (2013) observed similar color changes for lupine and soy bean, respectively.

According to ANOVA results, germinated seeds and germination period factors affected the ash, protein, phytic acid and total phenolic content of GSF ($P < 0.01$) significantly. Ash content of GSF varied between 1.26 and 2.80%. GGLF possessed higher (2.80%) ash content than the other GSF while the lowest ash values determined with GWF. Increasing germination period increased the ash content of GSF and the biggest change in ash content was observed after 5 day of germination. GGLF showed the highest protein content among the samples. This is due to its high protein content of green lentil compared to the other grains. During germination, increasing in protein content was observed. This result is in accordance with the study of Rosa-Millán et al (2019) who reported the significant increase in protein content of germinated bean compared to ungerminated bean. Total phenolic contents of GSF changed between 2056.38 mg GAE kg⁻¹ (for GWF) and 3377.00 mg GAE kg⁻¹ (for GGLF).

After 5 day germination period significantly higher total phenolic content of GSF (57.17%) was observed in comparison to ungerminated seed. Tarzi et al (2012) described that the phenolic compounds of acetone, hexane and methanolic extracts of germinated chickpeas were increased as 53.7, 60.1 and 92.8% during the germination, respectively. During germination marginal increase (104.95%) was observed with GGLF (data not shown). In a study of Zilic et al (2014) total phenolic content of 5 day germinated wheat sample was increased from 1431 mg GAE kg⁻¹ to 1627 mg GAE kg⁻¹. Also an increase in total phenolic content in germinated rye sample compared to raw rye sample reported before by Katina et al (2007). Yeo & Shadidi (2017) found that the total phenolic content of lentil seed as 6.75 GAE mg g⁻¹.

Table 1-Effects of germinated seeds and germination period on the color and nutritional properties of germinated seeds¹

Properties	Germinated seeds flour			Ungerminated seed (mean)	Germination period (day)		
	GWF ²	GRF	GGLF		1	3	5
L*	82.73 ^a	79.31 ^b	79.50 ^b	83.40 ^a	83.12 ^a	80.61 ^b	74.92 ^c
a*	1.33 ^b	2.11 ^a	-0.75 ^c	0.14 ^d	0.85 ^b	0.56 ^c	1.78 ^a
b*	12.33 ^c	13.65 ^b	21.39 ^a	14.60 ^c	15.61 ^b	15.66 ^b	17.29 ^a
SI ³	12.41 ^c	13.81 ^b	21.43 ^a	14.66 ^c	15.73 ^b	15.75 ^b	17.41 ^a
Hue angle	83.68 ^b	81.09 ^c	92.05 ^a	85.99 ^{ab}	85.78 ^b	86.85 ^a	85.83 ^a
Ash (%)	1.26 ^c	1.83 ^b	2.80 ^a	1.75 ^c	1.99 ^b	2.05 ^b	2.07 ^a
Protein ⁴ (%)	13.46 ^b	13.00 ^c	22.67 ^a	15.31 ^c	17.16 ^a	15.98 ^b	17.05 ^a
TPC ⁵ (mg GAE kg ⁻¹)	2056.4 ^c	2665.0 ^b	3377.0 ^a	2185.2 ^d	2347.2 ^c	2817.8 ^b	3434.3 ^a
Phytic acid (mg 100 g ⁻¹)	865.8 ^b	654.2 ^c	963.2 ^a	1250.9 ^a	1136.1 ^b	593.4 ^c	330.5 ^d
Ca (mg 100 g ⁻¹)	71.92 ^c	84.89 ^b	93.65 ^a	74.39 ^d	81.62 ^c	84.24 ^b	93.68 ^a
Mg (mg 100 g ⁻¹)	132.57 ^c	160.39 ^a	145.07 ^b	110.23 ^d	151.06 ^c	156.79 ^b	165.95 ^a
K (mg 100 g ⁻¹)	319.49 ^c	521.17 ^b	1016.26 ^a	562.98 ^d	611.97 ^c	656.09 ^a	644.85 ^b
P (mg 100 g ⁻¹)	264.53 ^c	358.01 ^b	432.38 ^a	316.62 ^d	350.58 ^c	373.24 ^a	366.13 ^b
Zn (mg 100 g ⁻¹)	1.92 ^c	3.05 ^b	3.38 ^a	2.17 ^d	3.05 ^b	3.20 ^a	2.72 ^c
Fe (mg 100 g ⁻¹)	2.23 ^c	3.17 ^b	6.58 ^a	3.54 ^d	3.94 ^c	4.20 ^b	4.30 ^a

¹, Means with same letter within row sharing a common letter are not significantly different (P<0.05). GWF, Germinated Wheat Flour; GRF, Germinated Rye Flour; GGLF, Germinated Green Lentil Flour; SI³, Saturation index 4 N×5.70 for cereal flours; N×6.25 for non-cereal flours; TPC⁵, Total Phenolic content.

Phytic acid contents of GSF changed between 654.15 mg 100 g⁻¹ (for GRF) and 963.21 mg 100 g⁻¹ (for GGLF). During germination phytic acid decreased and the lowest phytic acid value was observed after 5 day germination period. This is due to the increasing activity of phytase enzyme during germination. At the end of the germination period of approximately 7-8 days, the complete phytate content disintegration is reported (Ashton & Williams 1958). In this study, the phytic acid reduction of GRF, GWF and GGLF were 65, 74 and 79% after 5 day germination, respectively (data not shown). The primary task of phytase in germinated grains is to provide inorganic phytate phosphate in the early stages of germination (Frolich et al 1988). The enzymatic hydrolysis of the phytate in seed has great prospects for phosphorus metabolism during the germination of cereals (Yamagata et al 1980). In another study performed by Ghavidel & Prakash (2007), it was shown that the phytic acid content of raw and germinated lentil samples were 0.197 g 100 g⁻¹ and 0.157 g 100 g⁻¹ respectively, and phytic acid content decreased during germination.

GGLF gave the highest calcium, potassium, phosphorus, zinc and iron content. The highest magnesium amount was observed with GRF during the germination period. Calcium, magnesium and iron content increased by increasing germination period. While potassium, phosphorus and zinc content of the GSF increased until the 3rd day of germination, but after 3 days, a decrease of these parameters was observed. Surface color of cookies played a key-role for baking properties to determine the consumer preferences. L*, a*, b*, SI (saturation index) and hue angle values of cookies made with GSF are given in Table 2. The average lightness value of the cookies made with GSF ranged from 63.81 to 67.48 (Table 2). As shown in Table 2, the use of GWF in cookie formulation resulted an increase in lightness and hue angle values compared to the other GSF. In cookie products, reducing lightness value could be expected due to caramelization and Maillard browning reactions that resulting in the darkening of product, generally (Manzocco et al 2000), but in this study, using GSF in the cookie formulation resulted in increase for lightness. Color variation of the cookies may be explained through surface smoothness. Purlis & Salvadori (2009) reported that smooth surfaces were more effective to enhance the lightness than shrinkage. According to these antecedents using GSF resulted in smoother surface than control cookies and the lightness of cookies was clearer and brighter. While the cookies made with GWF resulted the highest lightness and hue angle values, the cookies made with GGLF gave the highest redness (a*),

yellowness (b^*) and saturation index values. The highest redness value of GGLF added cookies might be due to the low moisture content of cookie samples of GGLF. Pérez et al (2013) reported that the high water content in cookie results the cookie take more time to reach the water activity value corresponding to the maximum Maillard reaction rate.

Table 2-Effects of germinated seed flours and addition level on color properties of cookie samples¹

Germinated seeds flour / Addition level (%)	L^*	a^*	b^*	SI (Saturation index)	Hue angle
Germinated seeds flour					
GWF ²	67.48 ^a	4.29 ^c	26.31 ^b	26.66 ^b	80.75 ^a
GRF	63.81 ^c	6.11 ^b	25.64 ^c	26.38 ^b	76.59 ^c
GGLF	66.11 ^b	6.32 ^a	27.30 ^a	28.06 ^a	77.07 ^b
Addition level (%)					
0	62.53 ^c	4.90 ^c	25.59 ^c	26.06 ^c	79.15 ^b
5	69.35 ^a	5.02 ^c	27.35 ^a	27.81 ^a	79.59 ^a
10	65.69 ^b	6.27 ^a	26.35 ^b	27.15 ^b	76.82 ^c
15	65.63 ^b	6.11 ^b	26.37 ^b	27.12 ^b	76.99 ^c

¹, Means with same letter within column are not significantly different ($P<0.05$); GWF², Germinated Wheat Flour; GRF, Germinated Rye Flour; GGLF, Germinated Green Lentil Flour

Significant increases ($P<0.01$) were observed with GSF addition in lightness, redness, yellowness and saturation index values of cookie samples. The highest lightness, yellowness and saturation index values were obtained with a 5% addition level while the highest redness values were obtained with a 10% addition level.

The results were presented in Table 3 and shown that the diameters, thickness and spread ratio values of the cookie samples supplemented with GSF varied between 47.10 and 47.24 mm, 7.82 and 7.97 mm, 5.93 and 6.02, respectively. It can be seen that there were a significant ($P<0.01$) increase in diameter of the samples with 10 and 15% addition level of GSF compared to control. The cookies made with GRF gave the highest thickness values while GRF addition resulted the lowest spread ratio values of cookies. The cookies containing GGLF and GWF gave the highest spread ratio values.

Table 3-Effects of germinated seed flours and addition level on textural properties of cookie samples¹

Germinated seeds flour / Addition level (%)	Diameter (mm)	Thickness (mm)	Spread Ratio	Hardness (g)	Fracturability (g)
Germinated seeds flour					
GWF ²	47.24 ^a	7.86 ^{ab}	6.01 ^a	3428.55 ^b	39.87 ^{ab}
GRF	47.23 ^a	7.97 ^a	5.93 ^b	3704.88 ^a	40.13 ^a
GGLF	47.10 ^a	7.82 ^b	6.02 ^a	3209.49 ^c	39.43 ^b
Addition level (%)					
0	47.05 ^b	7.89 ^a	5.96 ^{ab}	3452.81 ^b	39.72 ^a
5	46.63 ^b	7.92 ^a	5.89 ^b	3774.91 ^a	39.84 ^a
10	47.53 ^a	7.80 ^b	6.10 ^a	3428.29 ^b	39.61 ^a
15	47.53 ^a	7.93 ^a	5.99 ^{ab}	3134.92 ^c	40.09 ^a

¹, Means with same letter within column are not significantly different ($P<0.05$); GWF², Germinated Wheat Flour; GRF, Germinated Rye Flour; GGLF, Germinated Green Lentil Flour

Data on the textural characteristics of cookies enriched with GSF are presented in Table 3. The tested cookies showed significant ($P<0.01$) differences according to GSF type and addition level of GSF with respect to hardness. The hardness of the cookies was found between 3209.49 and 3704.88 g and the fracturability values were observed between 39.43 and 40.13 g. The lowest hardness and fracturability value of the cookies was observed for cookies containing GGLF, while the GRF addition resulted in the highest hardness and fracturability values. Wani et al (2012) reported that, high hardness values are related to protein content, high protein content resulted in a more hard structure and this result was due to the interaction between protein and starch. Another is the fiber content that affects the texture.

GRF addition decreased the spread ratio and increased the hardness of cookies that may be due to dilution of gluten or less protein content of rye compare to the other seeds and less water availability for hydration. As the addition level of GSF increased in cookies, fracturability values were not affected while hardness of cookies increased at 5% addition level and decreased at higher levels. It is thought that the fluctuations in the hardness values are caused by the differences in the moisture content of the cookie samples.

Baranzelli et al (2018) noted that the increase in the germination time reduced tenacity and increased extensibility of the flours. Also we know that tenacious gluten composites were used to make bread, whereas extensible gluten composites were used for cake and cookie production (Melini et al 2017; Sanchez-Garcia et al 2015). So that addition of GSF is more suitable for cookie making than bread making.

The moisture and ash values of cookies made with GSF were given in Figure 1. The moisture value of the cookie samples were between 2.30% and 5.42%, the average was $3.90 \pm 1.23\%$. The cookies made with GRF gave the highest moisture content. The usage of GGLF gave the lowest moisture content. Cornejo & Rosel (2013) mentioned that germinated rice flour gave lower moisture content than raw rice flour. The ash amounts of the cookie samples varied between 1.55 and 1.73%. The values of ash did not significantly ($P > 0.05$) varied among the three GSF. The results showed that an increase was observed in the ash values according to addition level of GSF. This result was associated to the increase in the relative proportion of minerals due to the loss of other seed storage reserves reported by Borek et al (2006).

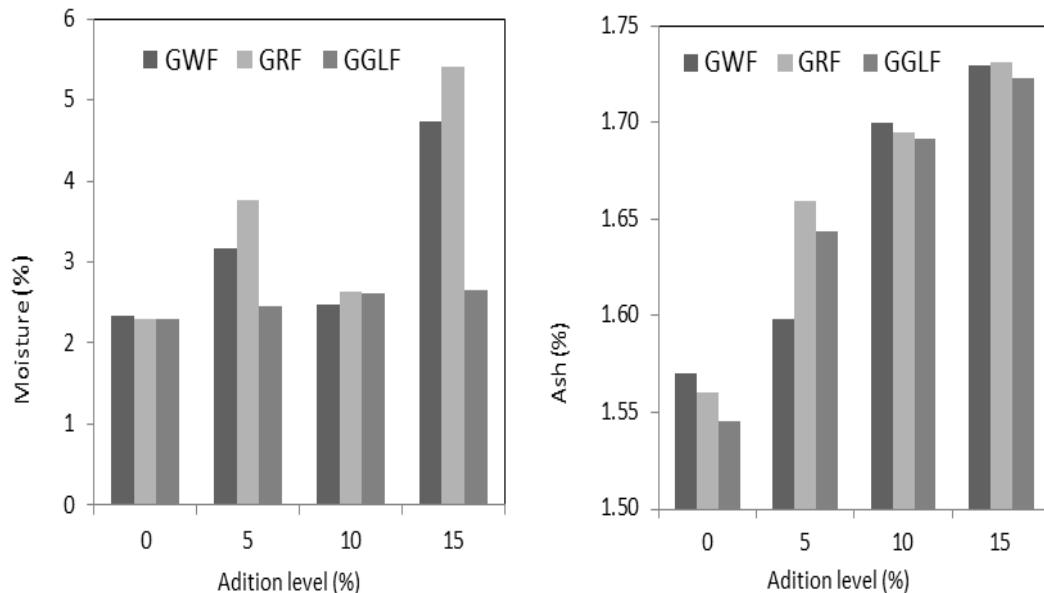


Figure 1- Moisture and ash contents of cookie samples (GWF, Germinated Wheat Flour; GRF, Germinated Rye Flour; GGLF, Germinated Green Lentil Flour)

Nutritional analyzes were performed at the end of the 5th day germination period and with only 15% substitution rate of the GSF added cookie samples. The effects of GSF addition on the phytic acid content of cookies are summarized in Table 4. The amount of phytic acid in the control cookie and GWF, GRF, GGLF added cookie samples were 222.60 ± 5.94 , 195.30 ± 2.97 , 203.70 ± 2.97 , 191.10 ± 2.97 mg 100 g^{-1} , respectively. The lowest amount of phytic acid was determined with GGLF and GWF added cookie samples, while the highest amount of phytic acid was obtained from control cookie sample (made with ungerminated wheat flour). Germination process decreases the phytic acid content due to the increasing phytase enzyme during germination. A comparable observation was described by Azeke et al (2011) who found that during germination, the level of phytase activity increased and reached its maximal value after six (5-fold), five (7-fold), and eight (6-fold) days of germination for maize, millet, and wheat and also on the seven (16- and 3-fold respectively) for rice and sorghum. Usually, legume based food product contain higher phytate contents than cereal-based food products. But in present study, GGLF added cookie samples gave lower phytic acid content than cereal based control cookie sample. Germination process is effective for decreasing the phytic acid content of legume based products.

Table 4-Effects of germinated seed flours on mineral contents of cookie samples¹

Germinated Seed Flours	Phytic Acid (mg 100g ⁻¹)	TPC ³ (mg GAE kg ⁻¹)	Calcium (Ca) (mg 100g ⁻¹)	Iron (Fe) (mg 100g ⁻¹)	Potassium (K) (mg 100g ⁻¹)	Magnesium (Mg) (mg 100g ⁻¹)	Phosphorus (P) (mg 100g ⁻¹)	Zinc (Zn) (mg 100g ⁻¹)
Control	222±5.94 ^a	713.5±6.36 ^d	32.04±0.02 ^d	1.54±0.03 ^b	122.23±0.08 ^d	19.66±0.02 ^d	557.16±0.72 ^a	0.84±0.01 ^c
GWF ²	195±2.97 ^c	838±4.24 ^c	41.42±0.01 ^b	1.47±0.01 ^c	146.27±0.17 ^c	31.62±0.03 ^b	507.17±0.63 ^c	1.01±0.01 ^a
GRF	203±2.97 ^b	904.5±4.95 ^b	54.50±0.01 ^a	1.04±0.01 ^d	162.49±0.36 ^b	39.05±0.01 ^a	303.64±0.66 ^d	0.95±0.01 ^b
GGLF	191±2.97 ^c	1095±4.24 ^a	36.71±0.01 ^c	1.83±0.02 ^a	195.07±0.28 ^a	26.97±0.01 ^c	559.90±0.90 ^b	1.00±0.02 ^a

¹, means with same letter within column are not significantly different (P<0.05); ², GWF: Germinated Wheat Flour; GRF, Germinated Rye Flour; GGLF, Germinated Green Lentil Flour; ³, TPC, Total Phenolic content

The total phenolic content (TPC) of cookie samples was presented in Table 4. Important phenolic compounds found in cereals; phenolic acids, flavonoids and tannins, which are concentrated in the bran fraction of cereal grains, are found in free, conjugated, and bound forms. Total phenolic content of control and GWF, GRF, GGLF added cookie samples were 713.50±6.36, 838.00±4.24, 904.50±4.95 and 1095.00±4.24 GAE mg kg⁻¹, respectively. TPC increased by 17.5, 27, 55% with GWF, GRF and GGLF addition in cookie formulation compared to control, respectively. As reported by Gawlik-Dziki et al (2016) after 4 days germination process, total phenolic content of wheat samples increased from 2.25 GAE mg g⁻¹ to 4.35 GAE mg g⁻¹. GGLF with rich TPC had a more dominant effect on TPC of cookie samples compared to the other GSF. Lentil has higher total phenolic and tannin content compared to other legumes. The TPC of lentils is thought to contribute significantly to the total antioxidant activity. The cotyledon of the lentil mainly contains non-flavonoid phenolic compounds, while the shell layer contains flavonoids.

Table 4 shows some mineral contents (Ca, Fe, K, Mg, P and Zn) in each of the cookies enriched with 15% level of GSF. Control cookie samples were determined as mg 100g⁻¹; 32.04±0.02 calcium, 19.66±0.02 magnesium, 122.23±0.08 potassium, 557.16±0.72 phosphorus, 0.84±0.01 zinc and 1.54±0.03 irons. The addition of GSF in cookie formulation increased the Ca, K, Mg and Zn content cookies compared to the control cookie sample. According to test results of cookie samples; the highest calcium and magnesium values were observed with cookies made GRF; the highest iron, potassium and zinc values were determined with GGLF cookies, while the lowest calcium, potassium, magnesium and zinc values were obtained from control cookie samples. The average daily mineral quantities are as follows: Calcium 1000 mg, iron 18 mg, potassium 3500 mg, magnesium 400 mg, phosphorus 1000 mg, zinc 15 mg. 100 grams of cookies made with GRF supplied 5.45% of the daily calcium content, 9.76% of the magnesium content, 4.64% of the amount of potassium, 30.36% of the phosphorus content, 6.33% of the zinc content, 5.78% of the amount of iron. 100 grams of cookies made with GGLF supplied 3.67% of the daily calcium content, 6.74% of the magnesium content, 5.57% of the amount of potassium, 55.99% of the amount of phosphorus, 6.67% of the zinc amount, 10.17% of the amount of iron.

Sensory analyzes were performed in cookies enriched with 0, 5, 10 and 15% GSF and shown in Table 5. The average organoleptic values of cookie samples evaluated between 1-5 points; the color values were 4.22-4.62, the appearance values were 4.23-4.45, the friability was 4.40-4.54, the taste was 4.10-4.57, the odor was 4.35-4.78 and the overall appreciation values were found between 4.32 and 4.51. The general appreciation of the cookies produced with the addition of GSF was evaluated as not different from each other. Generally, the highest appreciation value was found in 5% GWF added cookies and control cookies. In particular, the GWF added cookie received the highest (P<0.05) score for friability and taste, and the lowest score for odor. The cookies made with the GGLF gave the highest color and odor values, while the lowest scores were received for appearance with GGLF. The highest friability score was determined in cookie samples with supplemented GWF, while the most appreciated taste scores were determined in these samples. The increasing addition level did not affect the appearance of cookies. Color is one of the most important visual characteristic of cookies that strongly influences consumer's choice. Color scores was found similar scores up to 10% substitution rate, whereas more than 10% addition level decreased color score. Taste values showed similar scores with the control cookie samples up to 5% addition level, adding more than 5% level, resulting in a decrease in taste scores. Odor scores decreased with increasing addition level. In general, the addition of GSF to 5% level resulted in general appreciation scores similar to the control cookie samples, which led to a decrease in the probability of more addition levels.

Table 5-Effects of germinated seed flours and addition level on sensorial cookie properties¹

<i>Germinated seeds flour / Addition level (%)</i>	<i>Color</i>	<i>Appearance</i>	<i>Friability</i>	<i>Taste</i>	<i>Odor</i>	<i>General appreciation</i>
Germinated seeds flour						
GWF ²	4.40 ^b	4.45 ^a	4.54 ^a	4.53 ^a	4.53 ^b	4.49 ^a
GRF	4.22 ^c	4.41 ^a	4.43 ^b	4.24 ^c	4.45 ^b	4.35 ^a
GGLF	4.58 ^a	4.23 ^b	4.45 ^b	4.38 ^b	4.68 ^a	4.45 ^a
Addition level (%)						
0	4.62 ^a	4.38 ^a	4.40 ^b	4.50 ^a	4.78 ^a	4.50 ^a
5	4.45 ^a	4.40 ^a	4.53 ^a	4.57 ^a	4.62 ^b	4.51 ^a
10	4.38 ^a	4.27 ^a	4.50 ^a	4.35 ^b	4.45 ^c	4.39 ^b
15	4.28 ^b	4.40 ^a	4.45 ^{ab}	4.10 ^c	4.35 ^d	4.32 ^c

¹, Means with same letter within column are not significantly different (P<0.05); GWF ², Germinated Wheat Flour; GRF, Germinated Rye Flour; GGLF, Germinated Green Lentil Flour

4. Conclusions

The GSF is an alternative source in cookie formulation for nutritional enrichment. In this study, chemical, nutritional and sensorial properties of cookies enriched with GSF were investigated. Germination improved the nutritional quality such as ash, protein, total phenolic content, minerals and decreased the anti-nutritional factor as phytic acid. According to sensorial evaluation, the addition of GSF to 5% level resulted in general appreciation scores similar to the control cookie samples. Result of this study revealed that more nutritious cookies can be produced by up to 5% addition level of GSF and this formulation of cookie can be beneficially affects the nutritive composition and also does not impair sensorial attributes of cookies.

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