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ORIGINAL PAPER

Effects of germination on the physicochemical and nutritional characteristics of lentil and its utilization potential in cookie-making

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Abstract

Lentil is an alternative gluten-free source with high protein content. In our study, lentil seeds were germinated to enhance the functional and antioxidant properties of lentil. The raw and germinated lentil flour was obtained from lentil seed and used in cookie production. The germination affected the physicochemical, functional, chemical and morphological properties, and pasting behavior of lentil flour. The results indicated that germination caused positive effects on ash and protein content, total phenolic content (TPC), antioxidant properties, oil absorption capacity, and water solubility index. However, germination caused a decrease in the total dietary fiber and starch content due to the activation of the enzymes during germination. SEM images of the germinated lentil flour proved the degradation of starch. Lentil (raw and germinated) flour cookies and wheat flour cookies (Control) were evaluated in terms of physicochemical, TPC, antioxidant properties, textural properties, and in-vitro glycemic index (eGI) value. Germinated lentil flour cookie exhibited the lowest hardness, lightness, yellowness values, the highest TPC and antioxidant activity, and it had the same effect on eGI with control cookie. Overall results indicated that germination can be used as a natural, sustainable, and cost-effective way to improve the functional, and antioxidant properties of lentil. Germinated lentil flour cookie may be considered as a functional food due to high protein content and antioxidant properties.

Keywords Lentil · Germination · Cookie · Functional food · Antioxidant activity · Estimated glycemic index

Introduction

The increase in consumer demand for healthy foods creates a huge market for functional foods [1]. Cookie is considered as one of the widely consumed bakery foods for all age groups. It has many attractive properties including long shelf life, appropriate price and accessibility. Generally, traditional cookies are prepared from wheat flour, sugar and oil/fat [2]. These ingredients are nutritionally poor due to low in protein and dietary fiber. Therefore the interest in enrichment of cookies with a protein source such as legume, millet, bean is growing [3, 4].

Lentils (*Lens culinaris* Medic.) are important protein sources having from 21 to 31%, and the value is higher than some legume and cereal [5]. It can be constituted as an alternative protein source. It is also rich in dietary fiber, B-complex vitamins and minerals. In addition, it has high antioxidant capacity resulting from phenolics and flavonoids [6–11]. Phenolic components and flavonoids have an important role in the prevention of diabetes mellitus, coronary heart disease, and colon cancer [1, 12]. Besides lentil is gluten-free, which increases its interest in utilization as a food ingredient for the celiac diet.

An effective traditional processing technique is germination of seed/grain, which is one of the suitable cost ways to boost antioxidant properties and improve nutritional quality. In addition to these changes in seed, the functional properties and mineral bioavailability of seeds can be increased and their sensory and technological properties can be changed by germination [13, 14]. With germination, endogenous enzymes of seeds are activated, therefore major reserve molecules are degraded. As a result a new cell is formed and the biochemical, morphological, pasting, nutritional and functional properties of the seed are changed [15]. The effect of germination on phenolic compounds, antioxidant

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properties, nutrients, and antinutritional factors have been widely studied, however, to the best of our knowledge information on the utilization of germinated lentil flour in gluten-free cookies is not available in the literature. In the present study, the effect of germination on chemical, physicochemical, functional, pasting, crystallinity, chemical structure, and morphological properties of lentil were determined. Lentil flours were obtained from raw and germinated lentils and they were used to produce gluten-free cookies. The effect of germination on the total phenolic content, antioxidant, textural, and expected glycemic index value of the lentil-flour cookies were investigated.

Materials and methods

Materials

Green lentil (*L. culinaris*) seeds were obtained from Yayla Ltd. (Balikesir, Turkey). Seeds were stored in air tight container at 4 °C until further use. Commercial wheat flour, shortening, ground sugar, salt, sodium bicarbonate and skim milk powder were purchased from a local market (Nigde, Turkey). All chemicals were of analytical grade unless stated.

Germination

The green lentil seeds were germinated according to the method of Yiming et al. [16] with slight modifications. The seeds were washed with distilled water. Then seeds were placed between moist papers lined in trays and the trays were covered with aluminum foil. The lentil seeds were germinated at 25 °C for 5 days. Every day, distilled water was sprayed on papers to keep seeds moist. The germinated seeds were dried at 40 °C for 18 h. The raw and germinated seeds were ground with a laboratory mill to pass a 212 µm sieve to obtain raw lentil flour (RLF) and germinated lentil flour (GLF) samples, respectively. The flour samples were stored at 4 °C till further analyses.

Proximate composition of lentil flour samples

Moisture, ash and total lipid content of the flour samples (RLF and GLF) were determined according to AOAC methods of 925.08, 923.03 and 945.16, respectively [17]. The protein content (conversion factor (N); 6.25) of the samples were determined using the AOAC method of 992.23 [17]. Total dietary fiber (TDF) content was measured using Total Dietary Fiber Kit (K-TDFR; Megazyme, Ireland) according to AOAC method 991.43 [17]. The starch content of the samples was analyzed using Total Starch Kit (K-TSTA; Megazyme, Ireland) according AOAC Method 996.11 [17].

Physicochemical and functional properties of lentil flour samples

Water activity of flour samples were determined using water activity meter (Novasina, Lachen, Switzerland). Bulk density (BD), water absorption index (WAI) and water solubility index (WSI) of the flour samples were determined according to Chauhan et al. [18]. Oil absorption capacity (OAC) was determined using the method of Abdul-Hamid and Luan Abdul-Hamid and Luan [19].

Pasting properties of lentil flour samples

Pasting properties of flour samples were determined using a Rapid Visco Analyzer (RVA 4500, Perten Instruments, Australia). STD 1 method was applied, in brief 4 g of sample (14% moisture, db) was mixed with 25 mL of water and the samples were held at 50 °C for 1 min, then heated from 50 to 95 °C at a rate of 12 °C/min, held at 95 °C for 2.5 min and cooled from 95 to 50 °C at a rate of 12 °C/min, and held at 50 °C for 2 min.

FT-IR analysis of lentil flour samples

FT-IR spectra of lentil flour samples were obtained using Fourier Transformed Infrared Spectrometer (FT-IR, Thermo Nicolet Avatar 370). Measurements were performed in the wavenumber range of 4000–400 cm^{-1} and each sample was scanned 32 times.

Crystalline structure of lentil flour samples

The crystalline structure of lentil flour samples were investigated using X-Ray diffractometer (Bruker AXS D8, Germany). The X-ray source was CuKa radiation (wavelength = 0.15405 nm), and operating conditions were 40 kV and 30 mA. Data were collected over the 2 h range from 5° to 40° at a scanning speed of 0.06 °/min and a step size of 0.02°. Peaks in the traces were analyzed using EVA software (version 3.00) for XRD. Relative crystallinity (RC) of the flours were also calculated using the software of the diffractometer.

Scanning electron microscopy (SEM)

Morphological properties of the lentil flour samples were analyzed using scanning electron microscope LEO, 440 SEM–EDX systems (Leica-Zeiss, DSM-960). Flour samples, coated with gold palladium (60:40, g/g) in auto fine coater JEOL-JFC-1600, were placed on aluminum stub, before analysis.

Cookie preparation

Cookies were prepared according to the method of AACCI 10.54 with a slight modification [20]. Firstly, to make a cream, shortening (40 g) was mixed with ground sugar (40 g) in a stand mixer (KitchenAid K45SSWH, St. Joseph, Michigan, ABD) at speed 3 for 3 min, scraping down every minute. Water (40 mL) and dry mixture (skim milk powder (20 g), salt (1 g) and sodium bicarbonate (1 g) was added to the cream, then mixing was continued at speed 3 for 1 min scraping down every 15 s. Then the flour (100 g) was added to form dough and the mixture was mixed at speed 2 for 30 s scraping every 10 s. After mixing, dough was sheeted to 6.5 mm thickness, and then cut to give circular shapes of 5 cm diameter. The cookie samples were baked in an oven (Korkmaz A493, Turkey) at 170 °C for 15 min. To better understand the effect of lentil flour (RLF) and germinated lentil flour (GLF) on cookie quality, cookie with only wheat flour was also prepared (Control cookie sample). The cookies were left to cool for 1 h and then they were packed in polyethylene ziplock bags and allowed to stand at room temperature until analysis.

Physical and textural properties of cookies

The physical properties of cookies were determined in terms of diameter (D), thickness (T) and spread ratio (D/T) using a digital caliper. The textural properties of cookies in terms of break strength were measured by Texture Analyzer (TA.XT Plus Texture Analyzer, UK) using with a three-point bending jig 24 h after baking. Each test was performed with test speed of 3.0 mm/s and strain value of 10.0%. The maximum force to break the cookies was calculated as N. Color properties of the flour and cookie samples were measured using with colorimeter (Konica Minolta CR 400, Japan) based on CIE color values (L^*, a^*, b^*). L^* indicates lightness–darkness and ranges from 100 to 0. $+a^*$ and $-a^*$ represents redness and greenness, respectively. $+b^*$ indicates yellowness, while $-b^*$ indicates blueness [21].

Total phenolic content (TPC) and antioxidant activity of the samples

Prior to the TPC and total antioxidant analysis, flour and cookie extracts were prepared according to the method of Molinari et al. [22] with slight modification. Ground samples (1 g) were mixed with 20 mL solvent (methanol (80): water (20), v/v) and the tubes were inverted for 8 h at 25 °C. Then the tubes were centrifuged at 1000×*g* for 10 min. Supernatants were collected and stored at − 20 °C till analysis.

TPC of the samples were determined according to Folin–Ciocalteu method described by Pasha et al. [23]. The results were expressed as mg gallic acid equivalent (GAE)/100 g. The total antioxidant capacity of the samples were determined using two different methods: ABTS˙ radical scavenging and DPPH radical scavenging according to Severcan et al. [24] and Alaşalvar and Çam [25], respectively. DPPH and ABTS radical scavenging ability of the samples were expressed as mmol Trolox/kg.

Estimation of in-vitro glycemic index value of cookie samples

The rate of in-vitro starch hydrolysis of cookies was determined using the method described by Kahraman et al. [26]. At first, the starch content of cookies were determined using Total Starch Kit (K-TSTA; Megazyme, Ireland) according AOAC Method 996.11 [17]. The cookie samples were defatted and ground to pass 212 µm prior to the analysis. Cookie samples was hydrolyzed with digestive enzymes (pepsin (Sigma, P7000), pancreatin (Sigma-Aldrich, P7545) and amyloglucosidase (3300 U/mL, Megazyme Int., Ireland)) at 37 °C, then, hydrolyzed starch content was measured at different digestion times (20, 60, 90, 120, 180 min). Total starch hydrolysis (%) was plotted against digestion time. The hydrolysis index was calculated as follows;

$$\mathrm{HI} = \frac{\text{Area under the curve of the sample}}{\text{Area under the curve of the reference sample (white bread)}}$$

The estimated GI value was calculated using the equation given by Goñi et al. [27].

$$GI = 39.71 + (0.549 \times HI)$$

Statistical analysis

The obtained data were expressed as mean. Statistical analysis was performed with IBM SPSS Statistics version 24.0 (SPSS Inc., Chicago, IL). Differences between means at the $p < 0.05$ significance level was determined by Duncan's test.

Results and discussion

Proximate composition of raw and germinated lentil flour

Table 1 shows the characteristics of raw lentil flour (RLF) and germinated lentil flour (GLF) samples. Ash contents of RLF and GLF were 2.4% and 2.7%, respectively and the difference between ash content of these samples was significant ($p < 0.05$). This might be due to the fact that the germination process caused a decrease in the total soluble solids content of the flour sample. Similar observations were also reported in the literature conducted using amaranth [18, 28], lentil

Table 1 Proximate composition, functional, physicochemical, TPC and antioxidant properties of RLF and GLF flour samples

Parameter	Flour samples	
	RLF	GLF
Ash (%)	2.4[b]	2.7[a]
Protein (%)	24.6[b]	26.8[a]
Fat (%)	1.10[a]	0.75[b]
Starch (%)	49.6[a]	43.4[b]
TDF (%)	19.4[a]	15.4[b]
BD (g/mL)	0.80[a]	0.75[b]
WAI (g/g)	1.61[a]	1.38[b]
OAC (g/g)	0.73[b]	0.90[a]
WSI (%)	21.9[a]	22.7[a]
a_w	0.45[a]	0.35[b]
*L**	84.58[a]	82.23[b]
*a**	− 2.31[b]	− 1.17[a]
*b**	16.45[a]	15.50[b]
TPC (mg GA/100 g)	149.5[b]	183.3[a]
DPPH (mmol Trolox/kg)	4.5[b]	5.5[a]
ABTS (mmol Trolox/kg)	7.8[a]	10.1[b]

Ash, protein, fat, starch and TDF values are given as dry basis

RLF raw lentil flour, *GLF* germinated lentil flour, *TDF* total dietary fiber, *BD* bulk density, *WAI* water absorption index, *OAC* oil absorption capacity, *WSI* water solubility index, a_w water activity value, *TPC* total phenolic content, *GA* gallic acid

[a,b]Mean values in the same row with different superscript letters differ significantly ($p < 0.05$)

[29] and rice [30]. Protein content of the RLF was 24.6% and it significantly ($p < 0.05$) increased with the germination process (GLF; 26.8%). The increase might be due to the new amino acid synthesis by enzymes during germination [3, 18].

The fat content of RLF was 1.10% (Table 1). The germination process caused a significant decrease ($p < 0.05$) in the fat content of lentil flour (GLF; 0.75%). During germination intrinsic enzymes (i.e. such amylase, lipase, galactosidase, fiber-degrading enzymes, etc.) are activated [31, 32]. The decrease in fat content was associated with lipolytic enzyme activity during germination as also stated by Chauhan et al. [18]. The starch content of GLF (43.4%) was significantly lower than that of RLF (49.6%). The decrease in the starch content might be due to the fact that the starch hydrolysis into simple sugars and dextrin by enzyme activation during germination [3]. The germination resulted in a significant ($p < 0.05$) decrease in the total dietary fiber (TDF) content of RLF (from 19.4 to 15.4%). Similar decreases in TDF content of germinated flour samples were also observed in the literature. Dueñas et al. [29] stated that the decrease in the TDF content of germinated lentil flour was related to the change in cellulosic glucose content with metabolic reactions during germination. Gunenc et al. [33] also observed a reduction in TDF during the germination of wrinkle brown lentil. This situation was related to the reduction in hemicellulose content with the change of structural carbohydrates during germination [33]. According to Singh et al. [14], the reason for the decrease in fiber content of sorghum flour during germination was the cell wall degradation with metabolic reactions. Germination initiates biochemical and physiological changes and causes compositional changes in the grain. The decrease in the fiber content may be attributed to its breakdown and utilization during germination.

Physicochemical and functional properties of raw and germinated lentil flour

Physicochemical and functional properties of lentil flour samples (RLF and GLF) are shown in Table 1. The water activity value (a_w) of foods has an important role in microbial growth. The a_w values suitable for the growth of most bacteria, yeast and molds are 1–0.87, 0.91–0.87, 0.87–0.65, respectively. No microbial growth is observed at a_w below 0.61. In our study the a_w value of lentil flour significantly decreased from 0.45 to 0.35 with germination ($p < 0.05$), and the flours can be accepted safe in terms of microbial risk.

Bulk density (BD) of RLF (0.80 g/mL) decreased significantly with germination (GLF; 0.75 g/mL). Similar results were also observed in the literature [30, 34, 35]. Chauhan et al. [18] and Singh et al. [14] reported decreases in the BD values of amaranth and sorghum flours during germination, respectively. They both related the decrease in BD (as a result of germination) to the change in particle heaviness as a result of carbohydrate/dietary fiber modification with germination.

Water absorption index (WAI) was significantly ($p < 0.05$) affected by germination (Table 1). The GLF had lower WAI (1.38 g/g), as compared to RLF (1.61 g/g). The decrease in WAI can be associated with free sugar formation from starch degradation during germination. Similar decreases in WAI of germinated brown rice and sorghum were also observed by Cornejo and Rosell [36] and Singh et al. [14], respectively. As stated above, during germination intrinsic enzymes are activated and due to the starch hydrolysis high levels of dextrins and fermentable sugars are produced [3, 31, 32]. These released sugars form cross-linkages between starch chains causes a reduction in starch swelling and water absorption [14, 36]. In addition to these, Sosa et al. [37] reported that the starch structure might affected to the WAI of flours.

Germination had a significant ($p < 0.05$) effect on the oil absorption capacity (OAC) of lentil flour (Table 1). The OAC of RLF and GLF were 0.73 g/g and 0.90 g/g, respectively. The enhancement in the OAC of lentil flour might be due to the increase in lipophilic amino acids during germination leads to hydrophobic interaction with lipids [14].

Similar increases in OAC of germinated foxtail millet flour was also reported by Sharma et al. [35]. The water solubility index (WSI) of lentil flour (RLF; 21.87%) increased slightly with germination (GLF; 22.66%). However, the increase was not significant.

Color properties of raw and germinated lentil flour samples

Color characteristics of lentil flour samples (RLF and GLF) were shown in Table 1. The L^* (lightness) values of RLF and GLF were 84.58 and 82.23, respectively and the difference between L^* values of these samples were significant ($p < 0.05$). Similarly b^* (yellowness) value of GLF (15.50) was significantly lower than that of RLF (16.45). On the other hand, α^* (redness) value of lentil flour increased significantly ($p < 0.05$) with germination (RLF: −2.31; GLF: −1.17). These results indicate that, the germination led to darker (lower L^*), less yellow (lower b^*) and less green-looking flour ($-\alpha^*$: greenness). The decrease in lightness (L^*) probably might be due to the increase in protein and phenolic compounds [3, 18].

Total phenolic content (TPC) and antioxidant activities of flour samples

The TPC and antioxidant properties (DPPH and ABTS radical scavenging activity) of lentil flour samples (RLF and GLF) were presented in Table 1. TPC of RLF increased significantly ($p < 0.05$) from 149.5 to 183.3 mg GA/100 g with germination. Similarly germination caused a significant ($p < 0.05$) increase in DPPH and ABTS antioxidant capacity; from 4.5 to 5.5 mmol Trolox/kg and 7.8 to 10.1 mmol Trolox/kg, respectively. Similar results were also stated in the literature. Jan et al. [3] and Polat et al. [38] observed increases in TPC values and antioxidant capacity of Chenopodium and green lentil during germination, respectively. The increase in TPC value is probably due to an increase in the cell wall-bound phenolic release as germination leads a rise in the activity of cell wall degrading enzymes [3]. As the phenolic compounds have antioxidant activity, the improvement of antioxidant activity can also be related to the increase in enzyme activity.

Pasting properties of raw and germinated lentil flour

The effect of germination on RVA pasting properties of lentil flour samples (RLF and GLF) were shown in Fig. 1. Peak, though, final and setback viscosity values of RLF were 792.5, 769.5, 1154.0 and 384.5 cP, respectively. Germination caused decreases in peak, through, final and setback viscosity values; 628.5, 550.0, 704.5 and 154.5 cP, respectively. Similar trend in reduction in peak, through, final and setback viscosity during germination in Amaranth was reported Chauhan et al. [18]. The peak viscosity is one of important properties of starch which exhibited swelling capacity of starch molecules during heating [39]. The main reason of the lower peak viscosity after germination is losing resistance to swelling of starch [40]. Additionally, the other reason of the lower viscosity after germination is the degradation of the starch by enzymatic activity during germination, as stated by several researchers [3, 18]. The decrease in peak viscosity with after germination was consistent with the decrease in starch content (Table 1; RLF: 49.6%; GLF: 43.4%) and SEM images of flour samples (Fig. 3). Additionally, the high peak viscosity of RLF may be associated with high water absorption capacity (1.61 g/g) as it affects swelling of starch granule [41]. Breakdown viscosity indicates starch paste resistance to shear force [42]. Breakdown viscosity of raw lentil flour increased from 23.0 to 78.5 cP with germination. Ghumman et al. [43] also observed higher breakdown viscosity in germinated lentil starch compared to lentil starch. According to Chinma et al. [41], high breakdown viscosity is associated with high peak viscosity. The setback and final viscosity values are related to aggregation of starch molecules and represented retrogradation ability of starch paste [44, 45]. The decrease in final and setback viscosity can be attributed to low tendency of aggregation of starch molecules during cooling because of the starch degradation during germination [44]. In addition, these pasting parameters are important properties in food industry which affected the texture quality of end-product [46, 47]. In our study, the decrease in final and setback viscosity of flour was consistent with the results of texture properties, i.e., cookie prepared with GLF had a lower hardness value than that of RLF. Germination did not significantly affect the gelatinization temperature of lentil flour samples ($p > 0.05$). The

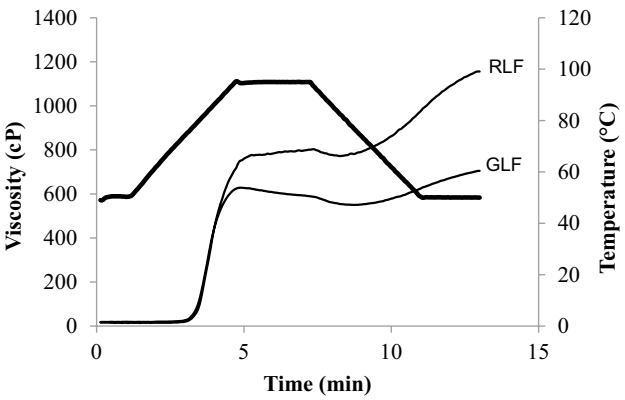

Fig. 1 RVA pasting curves of lentil flour samples. *RLF* raw lentil flour; *GLF* germinated lentil flour

gelatinization temperature of RLF and GLF were 79.4 °C and 78.3 °C, respectively.

X-ray diffraction patterns of raw and germinated lentil flour

The X-ray diffraction patterns of lentil flour samples (RLF and GLF) were shown in Fig. 2a. Both flours had a typical C-type crystalline structure with peaks at 15°, 17°, and 23° (2θ) [48]. The difference between X-ray diffraction patterns of the raw and germinated flour was not significant ($p > 0.05$), on the other hand the relative crystalinity value (RC %) of RLF decreased from 62.0 to 44.4% with germination. Similar results were also reported by Li et al. [44] who investigated the germination effect on different starches including brown rice, oat, sorghum, and millet. The decrease can be attributed to the degradation of starch due to the increased enzyme activity during germination [44].

FT-IR spectroscopy of raw and germinated lentil flour

FT-IR spectra of lentil flours (RLF and GLF) were shown in Fig. 2b. Both flours had peaks at 3700–3000 cm^{-1} which resulted from the stretching vibration of the hydroxyl group of the water molecule [24]. The absorption region at 3000–2800 cm^{-1} is related to C–H stretching due to CH_2 groups and the narrow region has shown to the presence of lipid in samples [49, 50]. The peaks at 1600–1700 cm^{-1} are amide I region which corresponds to stretching of –CN groups of protein [50]. In our study, the –CN absorption peak has changed from 1639 to 1636 cm^{-1} for RLF and GLF, respectively. The peaks at 1539 cm^{-1} can also be attributed to the stretching of –CN and –NH groups of proteins. The band between 1340 and 1400 cm^{-1} was related to stretching OH groups of water [49]. The absorption band at 1020–1158 cm^{-1} has indicated stretching of C–O and aliphatic C–N [51]. The peaks between 800 and 1200 cm^{-1} related to starch structure [50]. The peaks at 1074 indicated to COC and CO bonds of the glucose rings [49]. The spectra region between 1000 and 1100 cm^{-1} is associated with the crystalline structure, amylose/amylopectin ratio [50]. The decrease in intensity of these bands indicates the reduction in the relative crystallinity of starch [44]. This result supports the starch relative crystallinity results (section 'X-ray diffraction patterns of raw and germinated lentil flour').

Granule morphology of raw and germinated flour

The scanning electron micrographs (SEM) of lentil flours (RLF and GLF) are shown in Fig. 3. SEM images of RLF have showed that the lentil starch granule had a smooth intact surface, spherical-oval shapes and the granules were surrounded by protein matrix (Fig. 3a) [52, 53]. In contrast, it was observed that the starch granule surface was destroyed and the continuous matrix including protein bodies started to be slightly lost with germination (Fig. 3b). Our results were in agreement with Frias et al. [53] who demonstrated the changes of surface and protein body in raw and germinated lentil starch. Additionally, some hollows were observed in surface of GLF (Fig. 3b). Similarly, Li et al. [44] observed pore in starch granule during germination and who reported that the increase in pore was caused from partial starch hydrolysis. According to Xing et al. [54], the enzyme's activity during germination consists of three stages. Firstly, the enzyme adsorbs onto the starch surface and starch hydrolysis begins. In the second stage, hydrolysis increases and many small holes form on granule surface, thus channels that allow to enzyme to spread into the granule center expand. In the final stage, the granule surface is degraded and changed by the catalytic enzyme action. In addition, the increase in granule porosity induces to change in molecular structure, thus the change led to a decrease in granule crystallinity [40]. In our results, the alteration in granule surface of lentil flour is consistent with the change in cryttallinity index measured by XRD. Also, the effect of germination on morphological

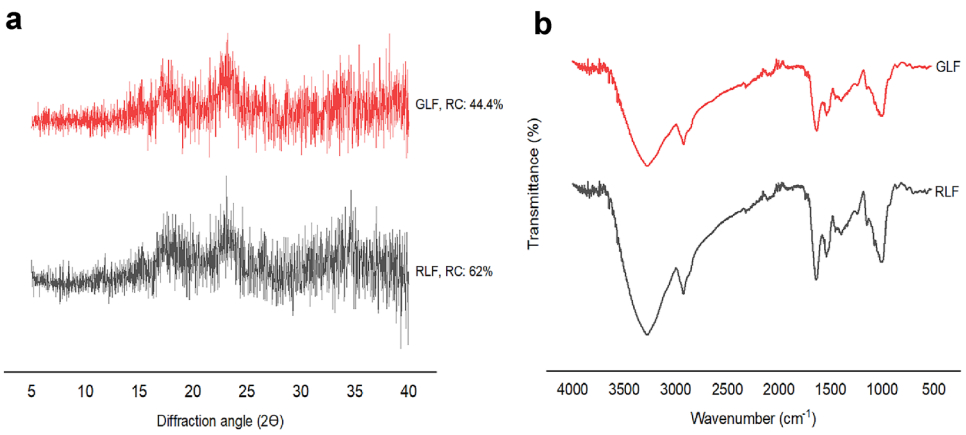

Fig. 2 **a** X-ray diffraction (XRD) patterns and **b** FT-IR spectra and of lentil flour samples. *RLF* raw lentil flour; *GLF* germinated lentil flour; *RC* relative crystallinity

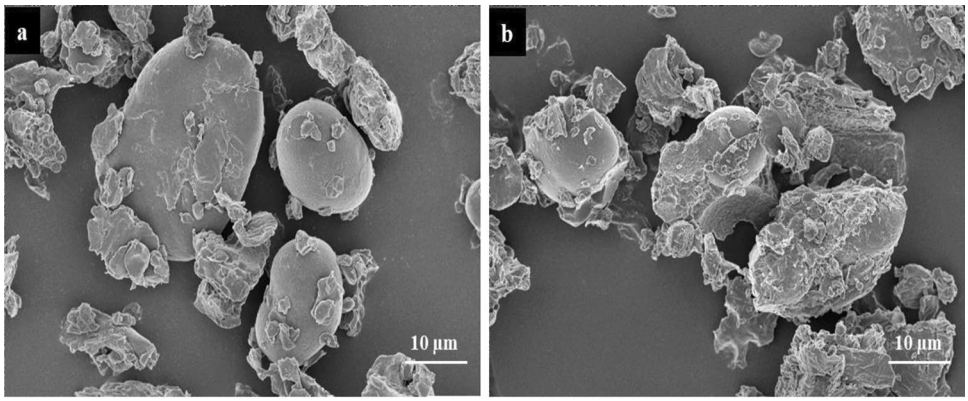

Fig. 3 Scanning electronic microscope (SEM) images of **a** RLF (Raw lentil flour), **b** GLF (Germinated lentil flour). Magnification 1.5 kX

properties was in agreement with previous studies showing the effect of the germination on different sources such as amaranth and Chenopodium [3, 18].

Color properties of cookie samples

Color properties of cookie samples prepared using only wheat flour (Control, C-WF), with raw lentil flour (C-RLF) and with germinated lentil flour (C-GLF) were shown in Table 2. C-GLF had the lowest L^* value among the samples (51.14). On the other hand, the highest L^* value (66.45) was recorded with the control cookie sample (C-WF). The b^* value of C-RLF was 29.44 and it significantly ($p < 0.05$) decreased in the C-GLF sample (22.39). As stated above (section 'Color properties of raw and germinated lentil flour samples') germination caused darker flour formation, therefore the cookie samples produced using germinated flour were darker (lower L^* and b^*). The images of the cookies were shown in Fig. 4. The decrease in the lightness (L^*) of cookies (also flours) might be attributed to the increase in protein and phenolic compounds [18]. Besides, brown melanoidin pigments formation during Maillard reaction also caused an increase in the darkness of the cookies [3]. It was evident that cookie sample prepared using lentil flours (RLF and GLF) had higher total phenolic content compared to wheat flour cookie (Table 2), and the lentil flour had higher protein content than wheat flour (Table 1; Wheat flour: 10.5%). The high phenolic and protein content in lentil flour may affect its color properties and, as a result lentil flours have higher browning effects on cookie than wheat flour. Jan et al. [3] also observed significant correlation between phenolic compounds and color properties. They reported that the high protein, sugar and phenolic content of C. album flour cookies resulted in darker cookies due to the Maillard browning reaction.

Table 2 Pysicochemical, textural, TPC, antioxidant and eGI properties of C-RLF, C-GLF and C-WF cookie samples

Parameter	Cookie samples		
	C-WF (control)	C-RLF	C-GLF
Moisture (%)	2.50^{c}	3.43^{b}	5.16^{a}
L^*	66.45^{a}	57.49^{b}	51.14^{c}
α*	4.46^{b}	5.52^{a}	5.64^{a}
b^*	34.11^{a}	29.44^{b}	22.39^{c}
Thickness (mm)	10.05^{a}	9.50^{a}	9.50^{a}
Diameter (mm)	63.5^{a}	53.00^{b}	52.5^{b}
Spread ratio	6.32^{a}	5.58^{b}	5.53^{b}
Hardness (N)	67.6^{a}	39.8^{b}	26.0^{c}
TPC (mg GA/100 g)	117.5^{c}	167.6^{b}	190.2^{a}
DPPH (mmol Trolox/kg)	2.6^{c}	4.9^{b}	5.7^{a}
ABTS (mmol Trolox/kg)	4.8^{c}	8.5^{b}	10.5^{a}
eGI	136.7^{ab}	124.7^{b}	144.0^{a}

C-RLF cookie prepared using raw lentil flour, *C-GLF* cookie prepared using germinated lentil flour, *C-WF* cookie prepared using wheat flour, *TPC* total phenolic content, *GA* gallic acid, *eGI* estimated glycemic index

$^{a-c}$Mean values in the same row with different superscript letters differ significantly ($p < 0.05$)

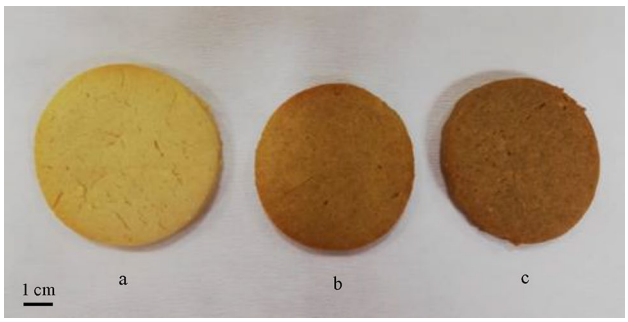

Fig. 4 Cookie samples prepared with **a** Wheat flour (C-WF, Control), **b** Raw lentil flour (C-RLF) and **c** Germinated lentil flour (C-GLF)

Physical, textural properties and moisture content of cookie samples

C-WF had the highest spread ratio (6.32, $p < 0.05$). However, there were no significant difference between the spread ratio values of C-RLF and C-GLF. Similar results were also reported in the literature. Simons and Hall III [4] observed that the difference in the spread ratio values of the cookie samples produced using raw and germinated bean flour was not significant ($p > 0.05$). In our study, the lower spread ratio of C-RLF and C-GLF cookies compared to the control cookie (C-WF) might be attributed to the protein content of flour. Both raw and germinated lentil flour had higher protein content than wheat flour (10.5%). According to Simons and Hall III [4], the protein content of flour plays an important role in the spread ratio. The increase in protein content of flour leads to higher water holding capacity, and thus, the dough becomes more viscous due to lateral flow restriction, and the spread ratio of cookie decreases.

The difference between the hardness values of the cookies were significant ($p < 0.05$) (Table 2). The highest hardness value belonged to control cookie (C-WF, 67.6 N). The hardness values of the cookies prepared with lentil flour were significantly lower than that of the control cookie ($p < 0.05$). Germination caused a significant ($p < 0.05$) decrease in the hardness value of the cookie sample. The hardness value of C-RLF was 39.8 N, and it decreased to 26.0 when GLF was used (C-GLF). Chauhan et al. [18] also showed a similar decrease in the hardness of cookies containing raw and germinated Amaranth flour. The decrease in the hardness value of the cookies was attributed to the formation of weaker cookie matrix due to starch degradation during germination.

The moisture content of the control cookie (C-WF) was 2.50%. Both cookie samples prepared with lentil flour had significantly higher moisture content than C-WF (Table 2). On the other hand, germination significantly influenced the moisture content of the cookies. The C-RLF had a moisture content of 3.43%, whereas the moisture content of C-GLF was significantly higher (5.16%). Similarly, Cornejo et al. [28] observed an increase in the moisture content of the cookie when germinated rice flour was used. According to them, this situation might be due to the fact that the macromolecules were breakdown during germination, causing an increase in the osmotic pressure and therefore the cookies retained more water. Keskin et al. [55] reported that the hardness of cookies was related to the moisture content and the higher moisture content contributed to softer texture. Chung et al. [2] have also reported that moisture content is one of the factors affecting the hardness of cookie. In our study, similar to the literature the hardness of cookies increased with a decrease in the moisture, as seen in Table 2.

Total phenolic content (TPC) and antioxidant properties of cookie samples

TPC and antioxidant properties (DPPH and ABTS radical scavenging activity) of the cookie samples are shown in Table 2. Control cookie sample (C-WF) had the lowest TPC, DPPH and ABTS radical scavenging activity (117.5 mg GA/100 g; 2.6 mmol Trolox/kg, 4.8 mmol Trolox/kg, respectively). The utilization of raw or germinated lentil flour in cookie formulation caused significant increase ($p < 0.05$) in the TPC and antioxidant activities values (Table 2). TPC, DPPH and ABTS radical scavenging activity of the cookie sample prepared using RLF (C-RLF) was 167.6 mg GA/100 g; 4.9 mmol Trolox/kg, 8.5 mmol Trolox/kg, respectively. As germination caused significant increases in the antioxidant properties of flour samples (section 'Total phenolic content (TPC) and antioxidant activities of flour samples'), the cookies prepared using GLF (C-GLF) had significantly higher TPC, DPPH and ABTS antioxidant activity (190.2 mg GA/100 g, 5.7 mmol Trolox/kg; 10.5 mmol Trolox/kg, respectively) compared to the C-RLF. Similar results were also observed in the literature. Jan et al. [3] observed that the utilization of germinated Chenopodium flour for cookie production led to an increase in the TPC and DPPH radical scavenging activity. Chauhan et al. [18] also reported that DPPH radical scavenging activity of germinated amaranth flour cookie was higher than that of non-germinated one. Similarly, Polat et al. [38] observed an increase in the ABTS radical scavening activity when germinated lentil extract was added to the formulation. In addition, baking process had a further enhancement on the TPC, DPPH and ABTS radical scavenging activity of cookie samples (Table 2) compared to flour samples (Table 1) due to formation of Maillard reaction products, as they have antioxidant properties [3, 18].

Estimation of in-vitro glycemic index value of cookie samples

The estimated in-vitro GI (eGI) was described postprandial incremental glycemic area after a meal and measured as the percentage of the corresponding area after an equi-carbohydrate portion of a white bread [27]. The estimated in-vitro GI (eGI) values of cookie samples were presented in Table 2. The eGI value of control cookie sample (C-WF) was 136.7. The lowest eGI was achieved with C-RLF (124.7), while germination significantly increased eGI value (C-GLC; 144.0). Cookie is a flour-based product that the major component is starch. Therefore, the change of starch granule of flour affects the textural, physical, digestibility of starch, eGI etc. of cookie. The increase in eGI of C-GLC resulted from change in starch properties during germination. Germination led to change in the crystalline structure of flour and the granule became more

sensitive to digestible enzymes [56]. This change in sensitivity to digestible enzymes of the flour was reflected in the glycemic index value of the final product. A similar result was observed by Frias et al. [53] in raw and germinated lentil starch. They reported that the rate of starch hydrolysis increased from 42 to 61% with germination. According to them, the difference in the hydrolysis rate between raw and germinated lentil starch samples might be attributed to the change in the crystalline structure of starch with germination. Bao et al. [56] has also observed a negative correlation between the eGI value and relative crystalinity. Similar results were also observed in our study. As stated above (section 'X-ray diffraction patterns of raw and germinated lentil flour'), the relative crystalinity of lentil flour decreased from 62.0 to 44.4% (Fig. 2a), while the eGI values of cookies prepared with RLF and GLF increased from 124.7 to 144.0 with germination (Table 2). Chung et al. [57] also reported that the germination of brown rice led to a decrease in resistance to digestive enzymes and an increase in starch digestibility. They observed that the amount of starch residual was less in germinated brown rice than un-germinated one, after 3 h of digestion. These results were attributed to the changes in starch structure and a decrease in crystallinity with germination [57]. de la Rosa-Millán et al. [58] has also observed a similar increase in the eGI of black bean with germination. The lower eGI in raw germinated lentil cookie (C-GLF) may be attributed to the protection of starch granule by dietary fiber. According to Reyes-Pérez et al. [59], dietary fiber reduces starch digestion by occurring a steric hindrance for digestive enzymes to reach starch. The decrease in the total dietary fiber content of flour after germination (Table 1) might result in higher eGI in cookie samples.

Conclusion

The rising trend on ''healthy living'' has significantly increased the consumer's demand for nutritious food. Germinated lentil was considered much healthier when compared to raw lentil due to anti-nutritional factors that are reduced during germination. The results of the present study showed that germination significantly affected the chemical, functional, morphological, crystallinity and pasting properties of lentil. Germination led to an increase in protein, total phenolic content and antioxidant activity, while caused a decrease in total dietary fiber. In addition, germination changed the pasting and functional characteristics and decreased the bulk density of lentil flour. The lower bulk density of germinated flour could be relevant when formulating complementary foods.

The bakery industry has been growing for hundreds of years by developing products such as cookie, bread, cracker which are the basic food products in human nutrition. It is important for celiac people to use various flours, that does not contain gluten, in cookie production. Germinated lentil flour cookie had the highest total phenolic content and antioxidant properties. Germination can be an advantageous way to improve some characteristics of lentil and utilization of germinated lentil flour in gluten-free cookies can be a nutritious alternative for celiac diet.

Declarations

Conflict of interest The authors declare no conflict of interest.

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