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Impact of Fermentation, Autoclaving, and Phytase Treatment on the Antioxidant Properties and Quality of Teff Cookies

Running head: Enrichment of cookies with dephytinized teff flours

İrem Karaçoban[1], Nermin Bilgiçli[1] and Elif Yaver[2*]

[1]Department of Food Engineering, Engineering Faculty, Necmettin Erbakan University, Koycegiz Campus, 42090, Konya, Turkey

[2]Department of Food Processing, Vocational School of Technical Sciences, Konya Technical University, 42250, Konya, Turkey

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SUMMARY

Research background. Teff [*Eragrostis tef* (Zucc.) Trotter] is an underutilized cereal crop that is cultivated mainly in Ethiopia and Eritrea. It is a great source of dietary fibers, vitamins, minerals, and bioactive compounds. However, it also contains a high amount of phytic acid, which is an antinutrient and reduces the bioavailability of minerals and proteins. To improve the nutritional quality of teff, the phytic acid content should be reduced by an effective dephytinization method.

Experimental approach. In this study, various dephytinization techniques (fermentation, autoclaving, and phytase treatment) were used to dephytinize teff flour. Undephytinized and dephytinized teff flours were incorporated into wheat flour (0−40 %) to enhance the functional properties of cookies. Twenty different cookie formulations were obtained according to (4x5)2 factorial design. Cookies were examined in terms of physical, chemical, nutritional, and sensory properties.

Results and conclusions. Among the dephytinization methods, fermentation elicited the most effective reduction in the phytic acid content (181 mg/100 g), followed by phytase-treatment (198 mg/100 g). The protein, fat, Fe, and Zn contents and antioxidant activity of cookies fortified with

*Corresponding author:
Phone: +903322232393
Fax: +903322410185
E-mail: elifyaver@hotmail.com

dephytinized teff flours were comparable to cookies fortified with undephytinized teff flour. Moreover, dephytinized teff cookies had lower phytic acid levels. The incorporation of 40 % teff flour exhibited stronger antioxidant properties and nutritional quality in cookies than the control wheat cookies. The use of dephytinized teff flours decreased the spread ratio, *a** and *b** values of cookies compared to undephytinized flour. Cookies containing fermented and phytase-treated teff flours elicited a harder texture than cookies containing undephytinized flour. Besides that, the increasing levels of teff flour gradually increased spread ratio values of cookies but decreased hardness. Overall acceptability scores of cookies containing 10-20 % teff flour were similar to the control.

Novelty and scientific contribution. To the best of our knowledge, this is the first study on the determination of the quality of cookies containing dephytinized teff flours. The data highlight the potential of dephytinized (especially autoclaved and phytase-treated) teff flours (up to 20 %) as functional ingredients to enrich the mineral content and antioxidant capacity of foods. Also, this study reveals that fermentation, autoclaving, and phytase treatment can be used to improve the nutritional quality of grains.

Keywords: autoclaving; cookies; fermentation; phytase enzyme; phytic acid; teff [*Eragrostis tef* (Zucc.) Trotter]

INTRODUCTION

Teff [*Eragrostis tef* (Zucc.) Trotter] is a gluten-free ancient cereal grain. Ethiopia and Eritrea are the main producers of teff. In the 2020/21 production year, 55,099,615.14 quintals of teff were produced in Ethiopia (*1*). Also, it is cultivated in India, Australia, USA, Canada, and South Africa (*2*). Teff has rich amounts of essential amino acids, unsaturated fatty acids, dietary fibers, vitamins, minerals (Ca, Fe, Mg, and Zn), and phytochemicals (*3,4*). Teff grain has great potential to prevent several diseases such as malaria, anemia, and diabetes (*2*). Due to its health benefits and nutritional profile, teff is gaining importance in recent years. It offers a good option for preparing functional baked and extruded foods (*5,6*). Ziec *et al.* (*7*) stated that the inclusion of teff flour at 5 %, 10 %, and 15 % levels enhanced protein, dietary fiber, Fe, Ca, Mg, and Mn concentrations of bread samples. Hager *et al.* (*8*) observed that the use of teff flour improved protein, ash, and dietary fiber amounts of gluten-free pasta in comparison with wheat pasta.

Despite health benefits, consumption of teff is limited due to anti-nutritional compounds including phytic acid (*5*). Phytic acid [myo-inositol (1,2,3,4,5,6)-hexakisphosphate] is the major phosphate storage compound in cereals, legumes, oilseeds, and nuts. It binds to minerals and proteins, thus reducing their bioavailability and digestibility (*9*). Hence, dephytinization methods such as fermentation, autoclaving, and phytase-treatment can be used to enhance the bioavailability of nutrients in teff (*5,6*). Fermentation is one of the simple ways to enhance the functional and nutritional characteristics of foods by increasing the levels of free amino acids, available vitamins, and minerals, and reducing the amounts of anti-nutritional compounds (*10*). Autoclaving is an inexpensive, simple, and green technique for reducing the phytic acid concentration in foods. Özkaya *et al.* (*11*) reported a reduction of about 95 % in the phytic acid content of oat bran by autoclaving for 90 min. Exogenous phytase treatment is another effective method of decreasing the phytic acid content. Garcia-Mantrana *et al.* (*12*) stated that the addition of phytase enzyme elicited high degradation (about 91 %) in the phytic acid level of bread.

Cookies are popular cereal-based products because of their availability, variety, practicability, low prices, and long shelf lives (*13*). However, the high fat and sugar content of cookies reduces their nutritional quality. With the increase in public awareness, the demand for healthy and nutritionally enriched cookies has increased (*14*). In this context, the inclusion of functional ingredients to improve the nutritional quality of cookies has been widely studied. Da Silva *et al.* (*13*) stated that *Spirulina maxima* biomass can be used as a protein- and iron-rich ingredient in cookie formulations without affecting sensory acceptance. Giuffre *et al.* (*15*) reported that olive oil can effectively replace shortenings to develop hardness, acidity, water activity values, unsaturated fatty acid content, antioxidant activity, and sensory quality of cookies. The incorporation of lupin flour, as a great source of proteins and dietary fibers, in enriched gluten-free cookies was studied by Csutoras *et al.* (*16*). In another study, Pinto *et al.* (*17*) revealed the potential of chestnut shells extract to produce acceptable functional cookies enriched with phenolics. Lagana *et al.* (*18*) prepared functional cookies with bergamot by-products and they found that fortified cookies have stronger antioxidant capacity than the control.

Teff flour has great potential to prepare functional cookies due to its unique nutritional characteristics. It was incorporated into cookies by several authors (*19,20*). Coleman *et al.* (*19*) investigated the impact of the use of teff flour (10-100 %) on the fracture strength, spread factor, and color values of cookies. Joung *et al.* (*20*) reported color, textural, sensory, and antioxidant properties of cookies containing 25 %, 50 %, 75 %, and 100 % teff flour. However, there are no studies on the inclusion of dephytinized brown teff flours into cookies.

To the best of our knowledge, this is the first study on the comparison of physical, textural, chemical, and sensory quality and antioxidant properties of cookies prepared from undephytinized and dephytinized teff flours. The objectives of this study were 1)to dephytinize teff flour using fermentation, autoclaving, and phytase treatment, 2)to develop nutritious cookie formulations with the incorporation of undephytinized and dephytinized teff flours at 0 %, 10 %, 20 %, 30 %, and 40 %

levels, and 3)to determine the effects of dephytinization methods and teff flour level on the phytic acid content, antioxidant properties, chemical, physical, textural, and sensory attributes of cookies.

MATERIALS AND METHODS

Materials

Brown teff seeds [*Eragrostis tef* (Zucc.) Trotter; Duru, Karaman, Turkey], powdered sugar (Kenton, İstanbul, Turkey), baking powder (sodium pyrophosphate and sodium bicarbonate; Dr. Oetker, İzmir, Turkey), salt (Cihan, Konya, Turkey), skimmed milk powder (Enka, Konya, Turkey), vanilla (Dr. Oetker), and baker's yeast (*Saccharomyces cerevisiae;* Pakmaya, Kocaeli, Turkey) were procured from local stores in Konya (Turkey). Wheat flour (*Triticum compactum* Host.; produced in 2020 and stored in glass bottles at 20±2 °C; Ova, Konya, Turkey) (contains 0.68 % ash, 10.14 % protein, 1.08 % fat, and 203 mg/100 g phytic acid) and all-purpose-type shortening (produced from palm oil in 2020; IFFCO, İzmir, Turkey) were obtained from a cookie factory located in Karaman, Turkey. Phytase enzyme (5000 FYT/g) was obtained from Novozymes (Bagsvaerd, Denmark). Dephytinization and cookie production experiments were conducted in 2020/21.

Methods

Dephytinization of teff seeds

Teff seeds were ground into whole meal using a laboratory mill (Arçelik-K3104, İstanbul, Turkey), divided into four parts and three parts of it were dephytinized through fermentation, autoclaving, or phytase treatment methods. Non-treated one part was called undephytinized teff flour.

Fermentation: Teff flour was mixed with distilled water at a ratio of 1:15 (*m/V*). The slurry was incorporated with 6% of yeast and fermented for 8 h at 30 °C in a water bath (Daihan Wisebath-WSB30, Gangwon, South Korea) (*21*).

Autoclaving: Teff flour and distilled water (1:3 *m/V*) were mixed. The pH of the slurry was set to 4.5 using acetic acid (Sigma-Aldrich, Steinheim, Germany). Then, the slurry was autoclaved (Daihan WiseClave Wac-60, Gangwon, South Korea) at 121 °C for 60 min (*11*).

Phytase treatment: Teff flour (100 g) was blended with 100 mL of 0.1 N acetic acid/sodium hydroxide buffer (pH 5.5; Sigma-Aldrich) and 0.5 g of phytase. The slurry was made up to 1000 mL with distilled water (pH 5.5). The slurry was shaken in a water bath (Daihan Wisebath-WSB30) at 37 °C for 2 h (*22*).

At the end of the dephytinization processes, the slurries were filtered and subjected to drying (Nüve-KD200, Ankara, Turkey) overnight at 50 °C. All the dried samples were ground using the laboratory mill (Arçelik-K3104).

Preparation of cookies

Cookies were made according to the AACC method 10-54 with some modifications (*23*). Cookies containing 0 % teff flour (control) were produced with 200 g wheat flour, 90 g powdered sugar, 80 g shortening, 4 g baking powder, 2.5 g salt, 2 g skimmed milk powder, 1 g vanilla, and water. The enriched cookies with teff flours were prepared by replacing wheat flour with undephytinized, fermented, autoclaved, and phytase-treated teff flours separately at 10 %, 20 %, 30 %, and 40 % levels.

All ingredients were mixed in a kneader (Hobart-N50, Ontario, Canada) for 5 min at low speed (level 1) until the homogeneous dough was obtained. The dough was sheeted to a height of 5 mm, cut into a 5 cm diameter circular shape, and baked (Vestel SF8401, Manisa, Turkey) at 175 °C, for 15 min.

Phytic acid

The phytic acid in the samples was extracted with 0.2 N hydrochloric acid (Merck, Darmstadt, Germany). The supernatant (0.5 mL) was reacted with 0.4 mM ammonium iron (III) sulfate (1 mL; Merck) and held in the boiling water bath for 30 min. Following that, samples were incubated in an ice bath for 15 min. After, 2 mL of 2,2'-bipyridine reagent (Merck) was added and absorbance at 519 nm was measured (Biochrom Libra-S22, Cambridge, UK) (*24*).

Antioxidant properties

For extraction, the samples (2 g) were reacted with 20 mL of acidified methanol (methanol:HCl:distilled water, 80:1:10 *V/V/V;* Merck) in the water bath (Daihan Wisebath-WSB30) at room temperature (25±2 °C) for 2 h. The extracts were centrifuged (1008x*g*, 10 min; Awel-MF20, Blain, France) (*25*).

For determination of total phenolic content, supernatant (0.1 mL) was mixed with 0.5 mL of Folin-Ciocalteu reagent (10 % *V/V;* Merck), 1.5 mL of sodium carbonate solution (20 %; Merck) and 7.9 mL of distilled water in a tube. The tube was held at room temperature for 2.5 h in the dark. The absorbance values at 760 nm were expressed as gallic acid equivalents (mg GAE/100 g) on dry weight basis using the calibration curve of gallic acid (Merck) (*26*).

For determination of antioxidant activity, the extract (0.1 mL) was reacted with 0.9 mL of 0.05 M Tris-HCl buffer (pH 7.4; Merck) and 2 mL of 0.1 mM DPPH solution (Merck). Blank was prepared with Tris-HCl+DPPH. The samples were kept at room temperature (30 min) and the absorbance was measured (Biochrom Libra-S22) at 517 nm. The percentage of inhibition was determined following equation (*27*):

$$\text{Antioxidant activity (\% inhibition)} = [(Abs_{Blank} - Abs_{Sample}) / Abs_{Blank}] \times 100 \qquad /1/$$

Chemical properties

Total ash content was measured in a muffle furnace (Daihan Wisetherm F12, Gangwon, South Korea) at 550 °C using the AACC method 08-01 (*23*). Crude protein level was determined by the Dumas combustion method using Leco FP828 (St. Joseph, MI, USA) according to AACC method 46-30 (*23*). Crude fat content was determined using the AACC method 30-25 (*23*).

For mineral analysis, samples were mineralized with sulphuric acid+nitric acid solution (Merck) in a microwave oven (Cem Mars-5, Matthews, USA). Ca, Fe, K, Mg, P, and Zn concentrations were determined by ICP-AES (Varian Vista AX, Zug, Switzerland) according to Skujins (*28*).

Moisture content was determined according to AACC method 44-19 (*23*).

Physical and textural properties

The diameter and thickness values were determined by a caliper (Mitutoyo, Tokyo, Japan) according to AACC method 10-54 (*23*). Spread ratio was calculated by dividing the diameter value by the thickness value of the cookies.

Cookie hardness was measured using a TA-XT.Plus texture analyzer (Stable Micro Systems, Surrey, UK) coupled with a three-point bending rig (HDP/3PB) (*23,29*). Pre-test speed, test speed, and post-test speed were set at 1.0 mm/s, 3.0 mm/s, and 10.0 mm/s, respectively. The test was made on at least five samples.

Color

Color L^* (lightness), a^* (greenness/redness), and b^* (blueness/yellowness) values were measured with a Minolta Chroma meter (CR-400, Osaka, Japan). Chroma (C^*) values were calculated from $C^*=(a^{*2}+b^{*2})^{1/2}$. Whiteness index (WI) was calculated following equation (*30*):

$$WI = 100 - [(100 - L^*)^2 + a^{*2} + b^{*2}]^{1/2} \qquad /2/$$

Determinations were made in five different positions on at least five samples.

Sensory properties

Sensory analysis was carried out by 12 experienced panelists (23-52 years old) among the staff of the Food Engineering Department of Necmettin Erbakan University. For the evaluation, the samples were placed in a plastic dish coded with random digits. Cookies were evaluated for color, taste, odor, appearance, and overall acceptability parameters, using a 7-point scale (1: dislike very much, 7: like very much). Drinking water was provided for palate cleansing (*31*).

Statistical analysis

The data of teff flours and cookies were compared by one-way and two-way analysis of variance (ANOVA), respectively, using Duncan's multiple comparison test. The values of $p<0.05$ were accepted significantly different. The data were the mean of triplicate determinations obtained from duplicate experiments and represented as mean±standard deviation.

RESULTS AND DISCUSSION

Phytic acid content, chemical properties and color values of teff flours

The phytic acid concentration of undephytinized and dephytinized teff flours changed between 181 mg/100 g and 1350 mg/100 g (Table 1). Maximum reduction (86 %) in phytic acid level of teff flour was obtained with the fermentation process, followed by phytase treatment (85 %) and autoclaving (69 %). The reduction in phytic acid level of fermented teff flour was potentially due to the action of endogenous phytase enzyme in teff flour and the phytase activity of yeast (*12*). The reduction in the autoclaved sample could be attributed to the endogenous phytase activity of teff flour and an increase in the solubility of phytate complexes under high temperature and pressure and low pH in autoclaving process (*4*). Özkaya *et al.* (*32*) found a decrease in the phytic acid content of rice bran by about 93.9% using autoclaving process at 121 °C and pH 4.5 for 60 min. The efficiency of phytase enzyme addition on the reduction of phytic acid level was also reported by Rosa-Sibakov *et al.* (*33*), who found a decrease of over 80 % in phytic acid concentration of faba bean by exogenous phytase (20 U activity) treatment (55 °C, 1 h).

All dephytinization methods caused a decrease in total phenolic content and antioxidant activity of teff flour (Table 1). The decrease could be originated from the leaching of phenolics into the water throughout the soaking and filtering steps in dephytinization processes (*21*). Özkaya *et al.* (*11*) reported similar decreases in phenolic contents of dephytinized oat bran samples by fermentation and autoclaving. However, antioxidant activities of dephytinized teff flours were statistically similar to each other ($p>0.05$) (Table 1). A previous study on dephytinization of cereal brans indicated that autoclaving method did not remarkably change the antioxidant activity of the samples compared to the control (*32*).

The ash amount of teff flours ranged from 1.72 % to 1.99 % (Table 1). Autoclaving and phytase treatment methods did not have a significant ($p>0.05$) effect on the ash level of teff flour. However, fermentation process decreased ash level of teff flour from 1.99 % to 1.72 %. The result might be associated with the leaching of soluble components during soaking and filtering processes in dephytinization processes or the metabolic activities of yeasts (*11*). Protein content of fermented (11.56 %) and autoclaved (11.34 %) teff flours was similar to undephytinized teff flour (11.46 %) (Table

1). However, phytase treatment resulted in a reduction in the protein amount of teff flour, probably due to dry matter loss during dephytinization (*11*). Fat contents of dephytinized teff flours were similar to undephytinized teff flour (Table 1).

As seen in Table 1, phytase-treated teff flour had the highest *L** value, and the lowest *a** and *b** values. The observations might be associated with the chemical profile of phytase-treated sample and the higher loss of pigments in teff during phytase-treatment process (*34*). On the other hand, the lowest *L** and the highest *a** and *b** values were observed in autoclaved teff flour (Table 1). Similar results were found by Rico *et al.* (*35*), who observed a decrease in *L** value and an increase in *b** value of wheat bran after autoclaving. They explained these changes in color by the occurrence of Maillard reaction products throughout the autoclaving process. *C** and WI values of teff flour samples ranged between 9.62-15.77 and 53.96-70.18, respectively (Table 1). *C** values of fermented and phytase-treated teff flours were lower than undephytinized flour. Besides that, WI value of undephytinized teff flour (60.90) increased after fermentation (64.81) and phytase-treatment (70.18). The increase in WI value may be originated from the loss of color pigments in brown teff flour during dephytinization processes (*34*). Surfiana *et al.* (*36*) also found an increase in WI value when fermented cassava flour. On the other hand, autoclaving process resulted in the highest *C** and the lowest WI values in teff flour, which was probably due to the formation of the Maillard reaction during autoclaving (*35*). Similar to the result obtained in this study, Espinosa-Solis *et al.* (*37*) observed that autoclaving process notably increased *C** value of malanga flour, possibly due to the development of non-enzymatic browning reactions.

Phytic acid content of cookies

The phytic acid levels of cookies are presented in Table S1, and the effects of dephytinization method and teff flour level factors on the phytic acid levels of cookies are shown in Table 2. The phytic acid levels ranged between 69.00 mg/100 g and 413.80 mg/100 g (Table S1). All dephytinization methods were effective in reducing the phytic acid level of cookies (Table 2). Compared to cookies made from undephytinized teff flour, the use of fermented and phytase-treated teff flour in cookies revealed about 70 % less phytic acid content. Besides that, the incorporation of autoclaved teff flour decreased phytic acid level of cookies by 53 % in comparison with cookies containing undephytinized sample. Baumgartner *et al.* (*21*) also reported a substantial decrease in phytic acid levels when 21 % dephytinized (fermented and autoclaved) oat bran samples were added to cookies.

Concerning teff flour level factor, increasing levels of teff flour gradually increased the mean phytic acid level of cookies (Table 2), which is mainly because of the high phytic acid level of undephytinized teff flour (Table 1). Köten (*38*) found a similar trend for phytic acid levels of tarhana fortified with teff flour at 0 %, 20 %, 40 %, 60 %, 80 %, and 100 % levels.

Antioxidant properties of cookies

Total phenolic content and antioxidant activity of cookies are compiled in Table S1 and Table 2. In terms of dephytinization method, the inclusion of dephytinized teff flours decreased the mean total phenolic content of cookies compared to undephytinized teff flour (Table 2). This is potentially due to the lower phenolic content of dephytinized teff flours than undephytinized teff flour (Table 1). However, dephytinization methods did not show an adverse effect on the antioxidant activity of cookies (Table 2). Similar observations were reported by Baumgartner *et al.* (*21*) for phenolic content and antioxidant activity after replacing undephytinized oat bran with fermented and autoclaved bran samples in cookies.

As shown in Table 2, the mean total phenolic content of cookies enhanced with the replacement level of teff flour increasing. It was probably caused by the fact that the teff flour contains a great amount of phenolics. The antioxidant activity of cookies containing 10 % teff flour was close to the control (0 % teff flour) (Table 2). Furthermore, the substitution of wheat flour with teff flour at higher levels (20-40 %) in cookies elicited stronger antioxidant activity than the control. The result could be attributed to the high antioxidant activity of teff flour (Table 1). Homem *et al.* (*4*) stated that bread made with 100 % teff flour had a greater antioxidant capacity and richer phenolic content than bread made with 100 % wheat flour.

Chemical properties of cookies

Ash, protein, and fat results of cookies are given in Table S2. The effects of dephytinization method and teff flour level on ash, protein, and fat contents are demonstrated in Table 3. Regarding dephytinization method, the inclusion of autoclaved teff flour revealed similar ash content in cookies to the samples made from undephytinized teff flour (Table 3). However, the use of fermented and phytase-treated teff flours slightly decreased ash content of cookies compared to cookies containing undephytinized flour. The results could be due to a slightly lower ash amount of fermented and phytase-treated teff flours than undephytinized flour (Table 1). On the other hand, there were no differences in protein and fat contents among cookies formulated with undephytinized and dephytinized teff flours (Table 3).

Regarding teff flour level, the addition of 40 % teff flour led to an enhancement in ash, protein and fat amounts of cookies compared to the control (Table 3). The findings could be due to higher ash, protein and fat contents of teff flour (Table 1) than wheat flour (0.68 % ash, 10.14 % protein, and

1.08 % fat). Thus, the data illustrated that the substitution of wheat flour with teff flour improves the nutritional profile of cookies. Our findings agree with the results of Köten (*38*), who obtained an increase in the ash, protein and fat concentrations after adding 40 % teff flour into tarhana.

Mineral composition of cookies

Mineral composition (Ca, Fe, K, Mg, P, and Zn) of cookies and effects of dephytinization methods and teff flour levels on mineral content are shown in Table S3 and Table 4, respectively. While cookies prepared from fermented and autoclaved teff flours had a slightly higher Ca level than cookies prepared from undephytinized flour, cookies containing phytase-treated teff flour showed the greatest Fe level (Table 4). The degradation of phytates during phytase treatment may be improved Fe bioaccessibility (*39*). On the other hand, Liang *et al.* (*40*) noted that the increase in minerals could be attributed to a proportional increase because of the loss of soluble compounds during soaking. Compared to cookies made from undephytinized teff flour, cookies made from dephytinized teff flours had lower K and P levels. The decreases in minerals may be because of the leakage of soluble solids during soaking and filtering treatments in dephytinization processes (*11*). Dephytinization methods did not show a significant ($p>0.05$) effect on Zn content of cookies.

In general, increasing levels of teff flour considerably enriched Ca, Fe, K, Mg, and P concentrations of cookies (Table 4), which showed the potential of teff flour in enhancing the mineral composition of cookies. Cookies containing 10 % and 20 % teff flour showed similar Zn content to the control, but Zn content of cookies containing 40 % teff flour was almost twice that of the control counterpart. These results may be originated from the richer mineral composition of teff flour than wheat flour (*6*). Ziec *et al.* (*7*) also reported increases in Ca, K, Fe, P, and Mg concentrations when bread was produced by replacing 15 % of wheat flour with teff flour.

Moisture, physical and textural properties of cookies

The moisture content of cookies ranged between 2.58 % and 4.71 % (Table S4). Cookies containing dephytinized teff flours had a slightly lower moisture content than cookies containing undephytinized teff flour (Table 5). Regarding teff flour level, moisture content of cookies decreased with increasing teff flour substitution. Coleman *et al.* (*19*) noted that teff flour does not have a great water absorption capacity.

The incorporation of dephytinized teff flours decreased diameter and spread ratio values of cookies compared to cookies containing undephytinized flour (Table 5). On the other hand, thickness of cookies slightly increased with the use of dephytinized teff flours (except for autoclaved flour) instead of undephytinized flour. The findings may be attributed to alterations in the chemical composition of dephytinized teff flours because of the heat treatments, enzymatic reactions and also the leakage of soluble compounds, which can affect the dough viscosity, during soaking and filtering steps in dephytinization processes (*41*).

In terms of teff flour level, increasing teff flour levels gradually increased diameter and spread ratio values of cookies (Table 5). The use of 10 % teff flour in cookies exhibited a similar thickness to the control. However, the inclusion of higher levels (20-40 %) of teff flour decreased thickness of cookies. The results could be attributed to lowering the gluten level in cookies with the addition of teff flour. In cookies, gluten acts as a binding agent and increases the viscosity of the dough (*42*). The dilution of gluten level, the disruption of gluten network and the decrease in dough viscosity due to the inclusion of gluten-free teff flour may be responsible for increases in diameter and spread ratio and a decrease in thickness of cookies containing teff flour (*21,43*). The study by Coleman *et al.* (*19*) also stated that spread ratio values of cookies increased with increasing levels of teff flour and suggested that the result was probably due to the low water absorption capacity of teff flour. Generally, great spread ratio values in cookies are desirable goals (*44*).

Texture analysis showed that the incorporation of fermented teff flour elicited the hardest texture in cookies (Table 5). On the other hand, cookies made with phytase-treated teff flour exhibited a harder texture than cookies made with undephytinized teff flour. Interactions between protein, starch, and water have an impact on the hardness of cookies (*43*). The differences in hardness values of cookies (Table 5) may be associated with the changes in the composition of teff flour during dephytinization processes.

The hardness of cookies gradually decreased with the increase of teff flour level, presumably due to the reduction in gluten level by the addition of teff flour. The less gluten network formation could be reduced hardness of cookies (*45*). Joung *et al.* (*20*) found similar results after replacing wheat flour with teff flour at 25 %, 50 %, 75 %, and 100 % levels to produce cookies.

Color values of cookies

The *L**, *a*,* and *b** values of cookies ranged between 46.87-74.25, 0.59-6.86, and 13.05-23.96, respectively (Table S4). As shown in Table 5, cookies containing phytase-treated teff flour elicited maximum *L** (59.47) and minimum *a** (4.37) values. Besides that, *L** values of cookies enriched with autoclaved teff flour were similar to cookies enriched with undephytinized teff flour. On the other hand, *a** and *b** values of cookies decreased with the use of dephytinized flours in comparison with cookies containing undephytinized teff flour. The decrease could be attributed to the leakage of soluble proteins and pigments during soaking and filtering steps in dephytinization processes, reducing the occurrence of Maillard reaction products in cookies containing dephytinized teff flour (*34*). Oyeyinka *et al.* (*45*) reported that cookies made with unfermented cassava flour had higher *a** and *b** values than cookies made with fermented cassava flour. Similarly, Baumgartner *et al.* (*21*) found a decrease in the *a** and *b** values of cookies when untreated oat bran was replaced with fermented or autoclaved bran samples.

Concerning teff flour level, the inclusion of teff flour decreased *L** and *b** values but increased *a** values of cookies (Table 5). The color changes may be originated from the native color of teff flour. Similar trends in color values were observed by Coleman *et al.* (*19*), who investigated the impact of teff flour addition on the color of cookies. Lu *et al.* (*14*) also reached a similar finding that cookies formulated with chickpea flour had lower *L** and *b** values and greater *a** values than the control. The authors pointed out that the higher protein level of chickpea flour would cause the occurrence of the Maillard reaction which resulted in a darker color in cookies.

Sensory properties of cookies

Due to their better technological attributes, cookies containing 0 % teff flour (control), undephytinized teff flour (10-30 %), autoclaved teff flour (10-30 %), and phytase-treated teff flour (10-30 %) were subjected to the sensory evaluation.

Color scores of cookies containing undephytinized and autoclaved teff flours at all levels were similar to the control (Fig. 1). While color scores of cookies made with 10 % and 20 % phytase-treated teff flour were close to the control, cookies containing 30 % phytase-treated flour had a lower color score than that of the control. Taste and odor scores of cookies formulated with undephytinized, autoclaved (except for 30 %) and phytase-treated (except for 30 %) teff flours were close to the control. The incorporation of teff flours up to 20 % in cookies exhibited close appearance scores to the control. However, the use of teff flours at 30 % decreased the appearance score of cookies compared to the control, presumably due to the darker color of cookies containing 30 % teff flour (Table 5). The addition of undephytinized teff flour at all substitution levels led to cookies with similar overall acceptability scores to the control (Fig. 1). Cookies made with 10 % and 20 % autoclaved and phytase-treated teff flours did not illustrate any negative impact on the overall acceptability. However, the usage of 30 % autoclaved and phytase-treated teff flours exhibited the lowest overall acceptability scores. Joung *et al.* (*20*) also stated that the use of teff flour in cookies at 25 %, 50 %, 75 %, and 100 % levels did not show an adverse influence on the overall acceptability score of cookies.

CONCLUSIONS

Data obtained in this study indicated that dephytinized teff flours can be considered promising functional ingredients since their rich amounts of ash and phenolics and low phytic acid levels.

Moreover, the replacement of undephytinized teff flour with dephytinized teff flour significantly reduced phytic acid level of cookies without adversely affecting protein, fat, Fe, and Zn contents. Among the dephytinization methods, fermentation and phytase treatment methods elicited the lowest phytic acid levels in cookies produced from teff flour. On the other hand, autoclaving process resulted in greater ash content in cookies made from teff flour than the other dephytinization methods. The inclusion of teff flour into the cookie formulation demonstrated notable enhancements in antioxidant capacity and mineral composition. Also, the incorporation of teff flour into cookie formulation exhibited acceptable physical properties. Cookies prepared from dephytinized teff flour had lower spread ratio values than cookies prepared from undephytinized flour. The hardest texture (3039.6 g) was observed in cookies enriched with fermented flour, whereas the autoclaving process revealed the softest texture (2530.6 g). Sensory evaluation showed that the use of undephytinized teff flour (up to 30 %) and autoclaved and phytase-treated teff flours (up to 20 %) could allow to prepare cookies with comparable sensory properties to the control. In conclusion, the findings suggest that it is probable to develop healthy and nutritious cookies using dephytinized (especially autoclaved and phytase-treated) teff flours (up to 20 %) with acceptable technological and sensory quality.

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CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

SUPPLEMENTARY MATERIALS

Supplementary materials are available at: www.ftb.com.hr.

AUTHORS' CONTRIBUTION

İrem Karaçoban is responsible for investigation, resources, and formal analysis. Nermin Bilgiçli is responsible for methodology, project administration, funding acquisition, conceptualization, supervision, writing - review and editing. Elif Yaver is responsible for investigation, resources, formal analysis, writing - original draft, writing - review and editing.

ORCID ID

İ. Karaçoban https://orcid.org/0000-0001-6431-9852

N. Bilgiçli https://orcid.org/0000-0001-5490-9824

E. Yaver https://orcid.org/0000-0002-2651-9922

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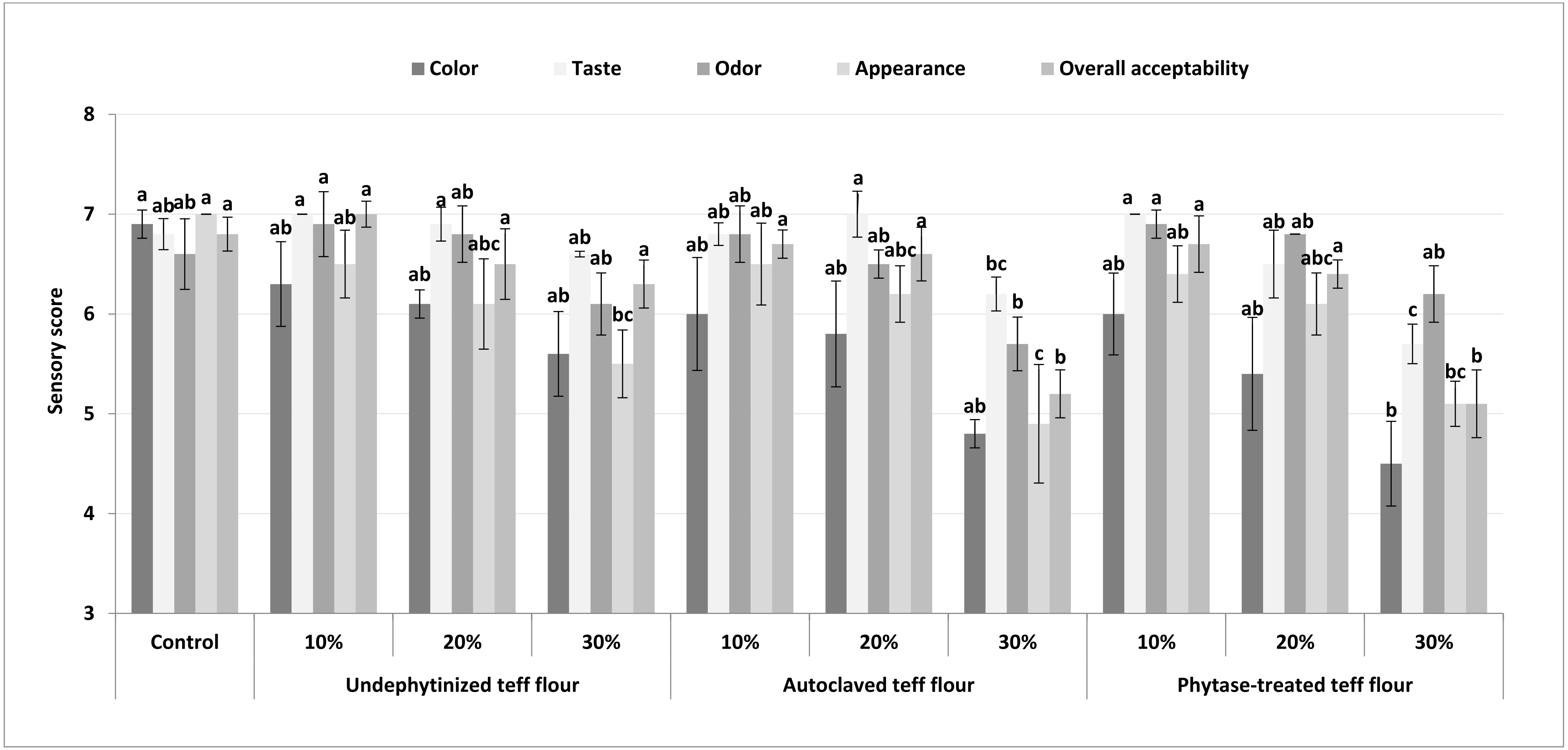

Fig. 1. Sensory evaluation of cookies

Table 1. Phytic acid content, antioxidant properties, macronutrients and color values of undephytinized and dephytinized teff flours

Parameter	Undephytinized teff flour	Fermented teff flour	Autoclaved teff flour	Phytase-treated teff flour
w(phytic acid)/(mg/100 g)	(1350±5.66)[a]	(181±1.41)[d]	(419±2.83)[b]	(198±1.13)[c]
w(TPC)/(mg GAE/100 g)	(547.06±7.09)[a]	(292.00±3.54)[b]	(256.00±2.69)[c]	(236.73±2.04)[d]
AA/%	(60.63±1.34)[a]	(40.25±1.98)[b]	(37.53±1.57)[b]	(37.08±1.51)[b]
w(ash)/%	(1.99±0.01)[a]	(1.72±0.04)[b]	(1.96±0.03)[a]	(1.89±0.03)[a]
w(protein)/%	(11.46±0.12)[a]	(11.56±0.13)[a]	(11.34±0.11)[a]	(10.87±0.10)[b]
w(fat)/%	(2.05±0.08)[a]	(2.03±0.10)[a]	(1.95±0.11)[a]	(2.12±0.06)[a]
Color				
*L**	(63.71±0.07)[c]	(66.57±0.10)[b]	(56.75±0.06)[d]	(71.77±0.07)[a]
*a**	(5.85±0.03)[b]	(4.45±0.02)[c]	(6.92±0.03)[a]	(3.40±0.05)[d]
*b**	(13.33±0.05)[b]	(10.04±0.08)[c]	(14.17±0.06)[a]	(9.00±0.07)[d]
*C**	(14.56±0.08)[b]	(10.98±0.05)[c]	(15.77±0.07)[a]	(9.62±0.06)[d]
WI	(60.90±0.07)[c]	(64.81±0.08)[b]	(53.96±0.06)[d]	(70.18±0.04)[a]

Means followed by the different superscripts within a row are significantly ($p<0.05$) different. The results (except for color) are based on dry weight. TPC=total phenolic content, AA=antioxidant activity, *C**=Chroma, WI=Whiteness index

Table 2. Effects of dephytinization method and teff flour level factors on phytic acid content and antioxidant properties of cookies

Factor	n	*w*(phytic acid)/(mg/100 g)	*w*(TPC)/ (mg GAE/100 g)	AA/%
Dephytinization method				
Undephytinized	10	(245.30±128.43)[a]	(128.02±32.47)[a]	(30.65±3.83)[a]
Fermented	10	(72.24±3.57)[c]	(108.14±20.31)[b]	(29.47±2.72)[a]
Autoclaved	10	(114.34±30.68)[b]	(111.68±16.75)[b]	(30.25±2.76)[a]
Phytase-treated	10	(72.34±3.35)[c]	(107.12±16.60)[b]	(30.06±2.40)[a]
w(teff flour)/%				
0 (Control)	8	(72.60±1.92)[e]	(88.54±3.33)[e]	(26.36±1.35)[c]
10	8	(98.83±36.83)[d]	(98.02±5.99)[d]	(28.64±1.78)[bc]
20	8	(128.23±77.26)[c]	(111.65±12.99)[c]	(30.51±1.35)[ab]
30	8	(154.15±114.98)[b]	(126.73±16.50)[b]	(31.75±1.51)[a]
40	8	(176.48±150.79)[a]	(143.78±16.72)[a]	(33.28±2.05)[a]

Means followed by the different superscripts within a column are significantly ($p<0.05$) different. Duncan's multiple comparison test according to two-way analysis of variance. Values are the mean of triplicate determinations obtained from duplicate experiments. The results are based on dry weight. n=number of samples analyzed, TPC=total phenolic content, AA=antioxidant activity

Table 3. Effects of dephytinization method and teff flour level factors on nutrient composition of cookies

Factor	n	*w*(ash)/%	*w*(protein)/%	*w*(fat)/%
Dephytinization method				
Undephytinized	10	$(1.67\pm0.09)^{a}$	$(5.64\pm0.14)^{a}$	$(21.28\pm0.44)^{a}$
Fermented	10	$(1.59\pm0.06)^{c}$	$(5.70\pm0.12)^{a}$	$(21.04\pm0.31)^{a}$
Autoclaved	10	$(1.67\pm0.11)^{a}$	$(5.66\pm0.10)^{a}$	$(21.12\pm0.32)^{a}$
Phytase-treated	10	$(1.62\pm0.09)^{bc}$	$(5.62\pm0.09)^{a}$	$(21.05\pm0.48)^{a}$
w(teff flour)/%				
0 (Control)	8	$(1.53\pm0.03)^{c}$	$(5.57\pm0.09)^{b}$	$(20.77\pm0.42)^{c}$
10	8	$(1.58\pm0.04)^{b}$	$(5.59\pm0.07)^{b}$	$(20.92\pm0.27)^{bc}$
20	8	$(1.63\pm0.05)^{b}$	$(5.66\pm0.08)^{ab}$	$(21.13\pm0.19)^{abc}$
30	8	$(1.71\pm0.07)^{a}$	$(5.71\pm0.10)^{ab}$	$(21.29\pm0.26)^{ab}$
40	8	$(1.75\pm0.06)^{a}$	$(5.76\pm0.12)^{a}$	$(21.52\pm0.31)^{a}$

Means followed by the different superscripts within a column are significantly ($p<0.05$) different. Duncan's multiple comparison test according to two-way analysis of variance. Values are the mean of triplicate determinations obtained from duplicate experiments. The results are based on dry weight. n=number of samples analyzed

Table 4. Effects of dephytinization method and teff flour level factors on mineral composition (mg/100 g dry weight) of cookies

Factor	n	Ca	Fe	K	Mg	P	Zn
Dephytinization method							
Undephytinized	10	$(43.88\pm5.81)^{b}$	$(1.72\pm0.38)^{b}$	$(131.84\pm22.81)^{a}$	$(38.68\pm9.27)^{a}$	$(272.04\pm30.34)^{a}$	$(0.60\pm0.21)^{a}$
Fermented	10	$(44.44\pm5.08)^{a}$	$(1.78\pm0.40)^{b}$	$(111.98\pm7.09)^{c}$	$(32.14\pm3.49)^{c}$	$(248.38\pm12.83)^{d}$	$(0.56\pm0.21)^{a}$
Autoclaved	10	$(44.74\pm5.96)^{a}$	$(1.66\pm0.30)^{b}$	$(120.56\pm16.75)^{b}$	$(38.78\pm7.98)^{a}$	$(268.36\pm28.90)^{b}$	$(0.64\pm0.17)^{a}$
Phytase-treated	10	$(42.16\pm3.98)^{c}$	$(2.16\pm0.66)^{a}$	$(111.32\pm8.21)^{c}$	$(33.24\pm4.14)^{b}$	$(252.06\pm15.64)^{c}$	$(0.64\pm0.21)^{a}$
w(teff flour)/%							
0 (Control)	8	$(36.90\pm0.79)^{e}$	$(1.30\pm0.11)^{d}$	$(100.45\pm2.30)^{e}$	$(27.58\pm0.66)^{e}$	$(230.90\pm2.23)^{e}$	$(0.43\pm0.10)^{b}$
10	8	$(40.20\pm0.84)^{d}$	$(1.50\pm0.09)^{d}$	$(108.60\pm4.48)^{d}$	$(31.00\pm1.95)^{d}$	$(245.78\pm5.81)^{d}$	$(0.48\pm0.11)^{b}$
20	8	$(44.05\pm2.41)^{c}$	$(1.85\pm0.25)^{c}$	$(121.15\pm10.95)^{c}$	$(36.03\pm3.49)^{c}$	$(259.68\pm12.32)^{c}$	$(0.55\pm0.06)^{b}$
30	8	$(47.55\pm1.66)^{b}$	$(2.13\pm0.26)^{b}$	$(128.58\pm14.98)^{b}$	$(40.83\pm5.15)^{b}$	$(275.85\pm17.18)^{b}$	$(0.75\pm0.14)^{a}$
40	8	$(50.33\pm2.10)^{a}$	$(2.38\pm0.49)^{a}$	$(135.85\pm15.93)^{a}$	$(43.13\pm6.78)^{a}$	$(288.85\pm21.22)^{a}$	$(0.85\pm0.12)^{a}$

Means followed by the different superscripts within a column are significantly ($p<0.05$) different. Duncan's multiple comparison test according to two-way analysis of variance. Values are the mean of triplicate determinations obtained from duplicate experiments. n=number of samples analyzed

Table 5. Effects of dephytinization method and teff flour level factors on moisture content, physical properties, hardness and color values of cookies

Factor	n	*w*(moisture)/%	*d*/mm	Thickness/mm	Spread ratio	Hardness/g	*L**	*a**	*b**
Dephytinization method									
Undephytinized	10	(3.82±0.58)[a]	(59.75±1.16)[a]	(5.90±0.51)[b]	(10.22±1.07)[a]	(2769.5±492.7)[c]	(58.45±9.51)[b]	(4.95±2.44)[a]	(18.79±3.39)[a]
Fermented	10	(3.55±0.25)[b]	(58.98±1.02)[b]	(6.21±0.48)[a]	(9.56±0.91)[b]	(3039.6±427.4)[a]	(57.43±10.37)[c]	(4.66±2.36)[b]	(17.24±3.91)[b]
Autoclaved	10	(3.24±0.51)[c]	(58.57±0.31)[d]	(6.04±0.38)[ab]	(9.72±0.56)[b]	(2530.6±567.5)[d]	(58.69±9.68)[b]	(4.56±2.26)[c]	(17.17±3.81)[b]
Phytase-treated	10	(3.25±0.57)[c]	(58.75±0.60)[c]	(6.21±0.31)[a]	(9.47±0.48)[b]	(3000.3±555.1)[b]	(59.47±9.36)[a]	(4.37±2.16)[d]	(16.72±4.13)[c]
w(teff flour)/%									
0 (Control)	8	(4.05±1.11)[a]	(58.16±0.18)[e]	(6.62±0.11)[a]	(8.78±0.17)[e]	(3575.8±22.8)[a]	(74.15±0.14)[a]	(0.60±0.01)[e]	(23.90±0.08)[a]
10	8	(3.70±0.66)[b]	(58.47±0.37)[d]	(6.34±0.31)[a]	(9.23±0.37)[d]	(3074.0±421.2)[b]	(62.68±0.68)[b]	(4.12±0.28)[d]	(18.59±1.22)[b]
20	8	(3.43±0.15)[c]	(58.90±0.78)[c]	(6.03±0.30)[b]	(9.79±0.56)[c]	(2792.3±344.3)[c]	(56.15±1.64)[c]	(5.62±0.45)[c]	(16.25±1.41)[c]
30	8	(3.19±0.43)[d]	(59.51±0.63)[b]	(5.81±0.19)[bc]	(10.24±0.38)[b]	(2463.6±216.4)[d]	(51.30±1.08)[d]	(6.25±0.35)[b]	(14.75±1.07)[d]
40	8	(2.98±0.35)[e]	(60.03±0.99)[a]	(5.65±0.32)[c]	(10.66±0.70)[a]	(2269.4±183.3)[e]	(48.26±0.93)[e]	(6.57±0.23)[a]	(13.91±0.84)[e]

Means followed by the different superscripts within a column are significantly ($p<0.05$) different. Duncan's multiple comparison test according to two-way analysis of variance. Values are the mean of triplicate determinations obtained from duplicate experiments. n=number of samples analyzed

Supplementary material

Table S1. Phytic acid content and antioxidant properties of cookies

Dephytinization method	*w*(teff flour)/%	*w*(phytic acid)/(mg/100 g)	*w*(TPC)/(mg GAE/100 g)	AA/%
Undephytinized	0	72.30±1.84	88.73±1.03	26.12±2.04
	10	156.70±2.40	100.20±3.96	28.12±3.00
	20	249.10±5.80	129.07±5.75	31.34±1.90
	30	334.60±6.22	152.70±3.82	32.41±2.25
	40	413.80±5.94	169.40±11.9	35.27±2.50
Fermented	0	73.20±3.11	88.40±3.39	26.00±1.41
	10	72.60±3.68	92.40±4.81	27.93±0.09
	20	73.90±5.52	99.73±6.69	29.54±0.65
	30	70.10±4.10	121.73±2.45	31.41±2.24
	40	71.40±5.09	138.45±2.05	32.48±2.14
Autoclaved	0	71.90±1.56	88.00±5.66	26.32±1.87
	10	92.80±3.11	103.40±4.81	29.18±2.57
	20	118.00±4.24	114.40±6.22	30.79±0.30
	30	137.30±3.82	118.07±4.34	32.17±1.17
	40	151.70±4.67	134.55±3.46	32.79±1.70
Phytase-treated	0	73.00±2.83	89.01±5.64	27.01±1.40
	10	73.20±2.55	96.07±7.17	29.31±1.86
	20	71.90±4.38	103.40±4.81	30.38±2.28
	30	74.60±0.57	114.40±3.68	31.01±1.40
	40	69.00±5.66	132.73±3.87	32.57±2.23

Values are the average of triplicate measurements on the duplicate samples. The results are based on dry weight. TPC=total phenolic content, AA=antioxidant activity.

Table S2. Nutrient composition of cookies

Dephytinization method	*w*(teff flour)/%	*w*(ash)/%	*w*(protein)/%	*w*(fat)/%
Undephytinized	0	1.55±0.03	5.56±0.16	20.79±0.13
	10	1.62±0.04	5.55±0.07	20.98±0.25
	20	1.67±0.03	5.62±0.11	21.20±0.28
	30	1.73±0.04	5.68±0.10	21.61±0.16
	40	1.79±0.01	5.79±0.24	21.84±0.20
Fermented	0	1.51±0.00	5.58±0.08	20.71±0.16
	10	1.55±0.06	5.58±0.07	20.89±0.34
	20	1.58±0.01	5.74±0.06	21.06±0.08
	30	1.63±0.04	5.79±0.14	21.15±0.21
	40	1.66±0.01	5.80±0.07	21.41±0.30
Autoclaved	0	1.53±0.03	5.56±0.10	20.82±0.25
	10	1.59±0.06	5.63±0.04	20.97±0.45
	20	1.65±0.07	5.66±0.08	21.25±0.21
	30	1.78±0.04	5.71±0.10	21.25±0.28
	40	1.79±0.01	5.76±0.08	21.31±0.40
Phytase-treated	0	1.52±0.04	5.57±0.10	20.76±1.06
	10	1.57±0.01	5.58±0.11	20.85±0.33
	20	1.61±0.06	5.63±0.04	20.99±0.16
	30	1.68±0.00	5.65±0.07	21.14±0.20
	40	1.74±0.01	5.69±0.13	21.50±0.25

Values are the average of triplicate measurements on the duplicate samples. The results are based on dry weight

Table S3. Mineral composition (mg/100 g dry weight) of cookies

Dephytinization method	*w*(teff flour)/%	Ca	Fe	K	Mg	P	Zn
Undephytinized	0	36.4±0.57	1.3±0.07	99.2±1.98	27.6±0.85	230.2±2.55	0.4±0.07
	10	40.8±1.13	1.4±0.03	115.3±1.84	30.5±0.71	250.4±1.56	0.5±0.07
	20	42.6±0.85	1.7±0.03	137.9±1.98	38.9±0.85	275.6±2.26	0.5±0.03
	30	47.2±0.28	2.0±0.14	149.6±0.85	45.6±0.85	294.8±2.55	0.7±0.08
	40	52.4±0.57	2.2±0.28	157.2±1.70	50.8±1.13	309.2±1.70	0.9±0.21
Fermented	0	37.6±0.85	1.2±0.07	103.2±0.99	28.3±0.42	232.4±3.39	0.4±0.14
	10	40.5±0.71	1.5±0.00	105.7±0.42	29.1±0.57	240.4±2.26	0.4±0.14
	20	45.6±0.85	1.9±0.07	113.6±1.56	31.4±0.57	245.1±1.56	0.5±0.07
	30	47.8±1.13	2.1±0.14	116.1±1.27	35.6±0.85	258.4±1.98	0.7±0.28
	40	50.7±0.99	2.2±0.14	121.3±0.99	36.3±0.42	265.6±2.26	0.8±0.14
Autoclaved	0	36.2±0.28	1.3±0.14	98.6±1.98	27.3±0.42	229.6±1.56	0.5±0.14
	10	40.3±0.42	1.5±0.14	108.3±1.84	33.9±0.57	251.7±0.42	0.5±0.21
	20	46.7±0.99	1.6±0.14	120.7±0.42	39.2±0.42	264.3±1.84	0.6±0.03
	30	49.6±0.85	1.9±0.07	132.9±1.56	45.6±0.85	288.5±2.12	0.8±0.07
	40	50.9±1.27	2.0±0.28	142.3±0.99	47.9±0.57	307.7±2.83	0.8±0.03
Phytase-treated	0	37.4±0.57	1.4±0.14	100.8±1.70	27.1±0.57	231.4±2.26	0.4±0.07
	10	39.2±0.28	1.6±0.03	105.1±1.56	30.5±0.71	240.6±1.98	0.5±0.03
	20	41.3±0.42	2.2±0.04	112.4±1.98	34.6±0.85	253.7±2.40	0.6±0.04
	30	45.6±0.85	2.5±0.14	115.7±0.99	36.5±0.71	261.7±1.84	0.8±0.14
	40	47.3±0.42	3.1±0.28	122.6±1.56	37.5±0.71	272.9±2.69	0.9±0.14

Values are the average of triplicate measurements on the duplicate samples

Table S4. Moisture content, physical properties, hardness and color values of cookies

Dephytinization method	*w*(teff flour)/%	*w*(moisture)/%	*d*/mm	Thickness/mm	Spread ratio	Hardness/g	*L**	*a**	*b**
Undephytinized	0	4.09±0.03	58.15±0.15	6.60±0.05	8.81±0.10	3571.0±14.0	74.15±0.15	0.61±0.01	23.92±0.07
	10	4.71±0.10	58.88±0.28	6.27±0.02	9.40±0.16	2910.5±8.5	62.14±0.21	4.51±0.04	20.51±0.11
	20	3.45±0.07	60.12±0.12	5.71±0.06	10.53±0.11	2585.0±5.0	55.42±0.19	6.22±0.03	18.41±0.06
	30	3.74±0.03	60.48±0.07	5.65±0.04	10.70±0.09	2622.4±22.4	51.73±0.09	6.54±0.01	16.25±0.13
	40	3.13±0.07	61.14±0.14	5.25±0.13	11.65±0.14	2158.5±18.5	48.84±0.05	6.86±0.05	14.83±0.03
Fermented	0	3.95±0.18	58.07±0.07	6.67±0.06	8.71±0.25	3565.0±5.0	74.04±0.09	0.62±0.01	23.87±0.02
	10	3.61±0.06	58.29±0.29	6.68±0.03	8.72±0.12	3359.7±19.7	62.47±0.15	3.86±0.06	18.24±0.01
	20	3.41±0.13	58.50±0.30	6.34±0.09	9.22±0.10	3116.4±3.6	54.06±0.38	5.76±0.06	16.02±0.07
	30	3.36±0.08	59.39±0.09	5.73±0.08	10.36±0.13	2655.0±10.0	49.73±0.12	6.60±0.03	15.04±0.08
	40	3.41±0.06	60.64±0.14	5.63±0.12	10.76±0.11	2502.0±13.0	46.87±0.08	6.45±0.01	13.05±0.08
Autoclaved	0	4.05±0.10	58.21±0.21	6.59±0.16	8.83±0.06	3559.0±6.0	74.25±0.04	0.60±0.00	23.84±0.02
	10	3.37±0.08	58.44±0.14	6.18±0.21	9.45±0.07	2510.6±8.4	62.42±0.12	4.20±0.03	17.99±0.21
	20	3.25±0.07	58.50±0.20	5.86±0.08	9.99±0.15	2373.6±6.4	57.22±0.20	5.28±0.07	15.65±0.10
	30	2.97±0.03	58.89±0.06	5.83±0.14	10.11±0.14	2148.0±11.0	51.29±0.10	6.05±0.04	13.85±0.07
	40	2.58±0.03	58.80±0.10	5.75±0.27	10.23±0.16	2062.0±13.0	48.26±0.23	6.65±0.05	14.53±0.08
Phytase-treated	0	4.10±0.08	58.19±0.19	6.62±0.07	8.79±0.10	3608.0±10.0	74.18±0.12	0.59±0.00	23.96±0.10
	10	3.09±0.11	58.26±0.26	6.23±0.36	9.35±0.30	3515.0±5.0	63.72±0.19	3.93±0.06	17.63±0.06
	20	3.59±0.08	58.48±0.08	6.20±0.19	9.43±0.12	3094.3±14.3	57.89±0.24	5.19±0.06	14.91±0.11
	30	2.67±0.03	59.27±0.07	6.05±0.07	9.80±0.09	2429.0±21.0	52.45±0.22	5.81±0.04	13.87±0.16
	40	2.78±0.16	59.53±0.13	5.95±0.04	10.00±0.23	2355.0±14.0	49.09±0.16	6.31±0.01	13.25±0.13

Values are the average of triplicate measurements on the duplicate samples