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Influence of Chia Seed, Buckwheat and Chestnut Flour Addition on the Overall Quality and Shelf Life of the Gluten-Free Biscuits

Running head: Novel gluten-free biscuits - An overall evaluation

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SUMMARY

Research background. In spite of being a significantly growing segment, there still exist problems regarding the nutritional, technological and sensory profiles of gluten-free products. Thus, the combination of a variety of functional ingredients is required in order to achieve the desired product quality.

Experimental approach. Flours of chestnut, buckwheat and potato were chosen in this study because they are all gluten-free; nutritionally richer and technologically more advantageous compared to wheat flour. They are applied together with chia seeds, which are also functional ingredients, due to being rich in dietary fiber and unsaturated fatty acids. Therefore, this study aims to evaluate the utilization of chia seeds with chestnut flour, buckwheat flour and potato flour in biscuits as overall quality enhancers in gluten-free products. The proximate composition, total phenolic content, antioxidant activity, some biscuit quality parameters and the sensory properties of the samples were investigated and some changes in these products during storage were monitored and evaluated.

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Results and conclusions. According to the results, biscuits with chestnut flour had the highest phenolic content (400.2 mg GAE/100 g dry sample) and total antioxidant activity (155.5 mg Trolox equivalent (TE)/100 g dry sample). Biscuits with chestnut and chia seeds had the hardness of 30.1 N ($p<0.05$). In addition, the use of chia seeds significantly increased the overall acceptability and flavor scores according to the sensory analysis results. During storage, chia seeds affected the oxidation stability, however the fatty acid profile stayed almost unchanged except for the losses in lauric acid, stearic acid and α-linolenic acid ($p<0.05$). In conclusion, the biscuits with chestnut and chia seeds were more prominent, with remarkably better nutritional characteristics and sensory attributes.

Novelty and scientific contribution. The study fulfills a need for the growing gluten-free market by incorporating the functional nutrients of chia seeds, chestnut flour and buckwheat flours together to achieve the production of nutritionally improved and organoleptically acceptable gluten-free biscuits. Furthermore, this study makes an overall evaluation of the changes in product quality during storage to provide new ideas for an overall innovation in the gluten-free foods market.

Key words: buckwheat, chestnut, chia, gluten-free biscuits, sensory characteristics, celiac disease, nutritional value

INTRODUCTION

Celiac disease is defined as the abnormal response of the immune system to wheat gluten and related prolamines of rye and barley (*1*). It mainly affects the small intestine as inflammation and causes a decrease in the absorption of some nutrients. It is medically defined and considered as being among the most common life-long disorders and a gluten-free diet is the recommended therapy for those patients (*1,2*). For another group, “perceived gluten sensitivity” also necessitates a gluten-free diet. Recently, the number of people suffering from chronic diseases has increased as a result of changing lifestyles. Accordingly, raising consumer awareness on the relationship between food and health has become the center of attention over the years. Thus, myriad consumers in addition to the celiac patients, prefer to consume gluten-free products to sustain a healthier lifestyle (*3*). Therefore, gluten-free food consumption has recently become an increasing trend for mainly these three different types of consumers with varying expectations (*4*). The current global market size is around 3,126 million US dollars and is expected to reach $5,279 million US dollars by 2022 with a steady growth (*5*). The growth may be explained by the increase in the number of celiac disease patients and diagnosed cases of wheat allergy and gluten sensitivity. However, from a sensorial and nutritional perspective, the gluten-free products are still a bit far away from meeting the demand of most consumers (*2*). Therefore, there is still a need for further research in order to better understand consumer expectations of gluten-free products.

Gluten is crucial in sustaining the viscoelastic properties of bakery products technologically; therefore, it is challenging to produce high quality and consumer acceptable gluten-free products. Consequently, research focused on numerous types of ingredients with non-gluten protein sources to enhance the overall quality in technological, sensorial, functional and nutritional aspects (*1,6,7*).

Nutritional properties of gluten-free products should be more in focus, as traditional gluten-free ingredients/flours are high in carbohydrates but low in proteins and antioxidants. Therefore, the use of dietary-fiber rich ingredients is proposed with a view to improving the nutritional quality of these products (*8,9*). Previous reports highlighted the higher propensity of celiac patients for excessive consumption of fat and sugar containing foods. This inclination could be a contributing factor to compensate for the limitations of gluten-free diet (*4*). Therefore, sustaining the nutritional profile of gluten-free products remains as a serious challenge, alongside their technical properties. Biscuits have significant attributes such as relatively long shelf-life, convenience and good eating quality and suitability for different nutritional/functional novelty trials, that make them good alternatives for gluten-free trials (*10*). Therefore, in this study, potato (*Solanum tuberosum*) flour with buckwheat (*Fagopyrum esculentum*) flour and chestnut (*Castanea sativa* Mill.) flour were used together with chia seeds (*Salvia hispanica* L.) (Lamiaceae) in gluten-free biscuit formulations, to enhance both nutritional and technological drawbacks.

Potato (*Solanum tuberosum*) flour is commonly used in gluten-free products because it is rich in essential amino acids (especially lysine), protein dietary fiber and it contains several phytochemicals (phenolics, flavonoids, and carotenoids) in addition to its high carbohydrate content (*11,12*).

Buckwheat (*Fagopyrum esculentum*) is a pseudocereal. Buckwheat flour, which is rich in catechins serving as antioxidants, also includes specific amino acids (such as lysine, histidine, valine and leucine), as well as minerals. Buckwheat proteins are mainly composed of albumins and globulins, with only a low amount of prolamins. In this respect, they are similar to leguminous proteins and considered as gluten-free (*13*). The dietary fiber is of great significance and together with the key polyphenols and potential antioxidant activities in buckwheat, it generally offers functional food quality due to its beneficial effects on health (*10,14*).

Chestnut (*Castanea sativa* M.) flour is popular in gluten-free formulations, due to its high protein quality with essential amino acids, high dietary fiber content and vitamins (such as vitamin E, vitamins B group) and minerals like potassium, phosphorous, and magnesium. Recent studies have evaluated the effects of supplementation of gluten-free biscuits and breads with different levels of chestnut flour (*2,15*).

The Chia plant (*Salvia hispanica* L.), originates from Central and South America (mainly southern Mexico and northern Guatemala), and particularly its seeds possess a technically unique property by means of which they can absorb large amounts of water to immediately form a transparent gel referred to as “chia mucilage”(*16,17*). This allows it to be used as a thickener, gel former, chelator, or fat replacer in different applications of the food industry such as bakery products. Chia seeds are nutritionally significant sources as they accommodate high levels of dietary fiber, protein and oil (particularly rich in polyunsaturated fatty acids (omega-3 fatty acids (α-linolenic acid, 54–67 %) and omega-6 (linoleic acid, 12–21 %)) and low in saturated fatty acids. They also possess other significant components such as tocopherols and phenolic compounds (chlorogenic acid, caffeic acid, myricetin, quercetin and kaempferol)(*18*).

Although some improvements have been achieved, the nutritional profile of gluten-free products still poses serious challenges despite their technical properties. In this context, this study aimed to evaluate the nutritional, technological and sensorial profile of gluten free biscuits and to monitor the product quality and changes during storage in order to develop an overall understanding of the products.

MATERIALS AND METHODS

Materials

Four different formulations: (1) Biscuit with buckwheat, 2) Biscuit with chestnut, 3) Biscuit with buckwheat and chia; and 4) Biscuit with chestnut and chia have been used for the production of gluten-free biscuits. For these formulations, buckwheat flour (Ecological Market Food Industry Trade Co., Turkey), chestnut flour (Naturelka CC Tourism Co., Turkey), potato flour (Sakarya Agricultural Products, Turkey), chia seeds (Güzel Ada Food, Turkey) lactose-free milk (Pınar Dairy, Turkey) were provided by the respective companies. In addition, the margarine, sugar and whole eggs were purchased from a local grocery store in Turkey.

Reagents

Ethanol (≥99.8 %), acetone (≥99 %) and copper (Delta Agricultural Chemicals Industry and Trade Inc.); sulfuric acid, sodium hydride, petroleum ether, hydrochloric acid, boric acid, potassium chloride, sodium bicarbonate, aluminum chloride and trifluoroacetic acid (Merck, Darmstadt Germany) in addition to gallic acid, 2,2 diphenyl 1-picrylhydrazyl (DPPH) and Trolox ((±)-6-Hydroxy-2,5,7,8-tetramethylchromane-2-carboxylic acid) from Sigma-Aldrich Co. (St. Louis, MO, USA) were used as chemicals.

Methods

Biscuit production

In this study, four different types of gluten-free biscuit formulations have been produced according to a modified method (*19*). The details are depicted in Table 1. The ingredients were mixed to form the dough and rolled to a thickness of 0.1 cm. Then the biscuits were molded with a cutter of 50 mm diameter and baked in a conventional oven at 150 ºC for about 25 min. Afterwards, they were left to cool down to room temperature for about 1 hour and then kept in airtight containers.

Nutritional evaluation

Proximate analyses

Total moisture, ash, protein and fat contents were determined according to the AOAC methods no: 925.10 (air oven method, gravimetric), (*20*), 923.03 (muffle furnace method, gravimetric) (*21*), 920.87 (titrimetric, Kjeldahl method) (*22*) and 945.16 (gravimetric, Soxhelet method) (*23*) respectively. The total amount of dietary fiber was determined using the Sigma Total Dietary Fiber Reaction Kit (TDF-100A; Sigma-Aldrich, USA), based on AOAC Method 985.29 enzymatic-gravimetric method (*24*). The total content of carbohydrates was calculated by the difference method, which involves adding the total amounts of moisture, protein, ash and fat constituents of the sample and subtracting it from 100. The value obtained is the percentage carbohydrate content of the sample (g/100 g). The total energy (kcal/100 g) was calculated using the conversion factors of 9 for each g of fats, and 4 for each g of carbohydrates and proteins.

Extraction for the determination of total phenolic content and antioxidant activity

A slightly modified extraction procedure was applied to prepare extracts for measuring the total phenolic contents and antioxidant activity (*25*). In summary, the samples (2 g) were first extracted by adding 15 mL of 80 % methanol and then shaken on an orbital shaker (Cole-Parmer, Model SSL1, Staffordshire, UK) for 15 min (4000×*g*), sonicated (Protech Mechanical Equipment Co, Ltd. Model PMUY-4L-D, Zhengzhou, China) for 15 min and centrifuged (Heittich-Rotofix 32A, Tuttlingen, Germany) at 4000×*g* for 20 min. The extraction was repeated until 30 mL of extract was collected in two replicates for each sample.

Total phenolics

Total phenolic contents of the samples were measured using the Folin-Ciocalteu method (*25*). According to the method, 750 µL of diluted Folin-Ciocalteu solution (1:10) was mixed with 100 µL of sample extract and 750 µL of sodium carbonate solution (6 %). After incubation for 90 min in the dark, the solution absorbance was measured at 750 nm against water with a spectrophotometer (Shimadzu V-1800, Kyoto, Japan). The analysis was repeated twice for each sample. Gallic acid was used as the reference and the results are given as GAE/100 g samples.

Total antioxidant activity (TAA)

The total antioxidant activity (TAA) of the biscuit samples was measured using the DPPH radical scavenging activity method (*26*). 1 mL of sample extract was added to a test tube containing 4 mL of methanol (80 %) and 1 mL of freshly prepared DPPH solution (containing 1 mmol DPPH), and the final concentration of DPPH solution was adjusted to 167 mmol. Then the tubes were kept in the dark for 30 min and sample absorbance was measured at 517 nm, taking Trolox as the reference.

Biscuit quality evaluation

The diameter and thickness of the biscuit samples were measured using a digital caliper. Bulk density was measured by dividing the mass of each sample to its volume. The spread ratio was measured by dividing the diameter (D) to the thickness (T). All measurements were made in triplicates. The volumes of the biscuits were measured using the rapeseed displacement method as described in AACC Method 10-05.01 (*27)* and water absorption test was performed according to the AACC Method 56-30.01 (*28*). In this method, ground samples (0.2 g) were weighed into pre-weighed centrifuge tubes and 10 mL of distilled water was added. The tubes were kept in 30 °C water bath for 30 min, vortexed for 5 s every 5 min and centrifuged at 3000×*g* for 10 min. The percentage of absorbed water was measured by subtracting the initial mass.

The color of the biscuit samples was measured using the color chromameter (CR-400, Konica Minolta Inc., Tokyo, Japan), using the Hunter scale of L^*, a^*, b^*. Six samples from each biscuit formulation were measured and the average was calculated. Color parameters for the standard white plane were as follows: L_o = 95.9, a_o = 0.2, b_o = 2.3.

The difference between the color parameters of darkness/lightness (L^* values), redness (a^* values), and yellowness (b^* values) for the biscuit samples and the standard white plane parameters given above, were calculated to measure the total color change (ΔE) according to the Eq. 1 below.

$$\Delta E^* = \sqrt{(L-L_o)^2 + (a-a_o)^2 + (b-b_o)^2} \qquad /1/$$

Hardness was detected using TA.XTplus Texture Analyzer (Stable Micro Systems, Surrey, UK), fitted with a 50-kg load cell and coupled with Texture Expert Software (Version 1.22; Stable Micro Systems) (*29*). Measurements were made approximately 24 hours after baking to measure the biscuit fracture, and each biscuit was compressed by a three-point bending rig (HDP/3PB) and blade (90 mm long and 3 mm thick) at a crosshead speed of 3 mm/s. The biscuits were 10 mm thick. The maximum force (N) measured in the force-time (distance) curve was taken as the value of hardness (*30*).

Sensory evaluation of the biscuits

Sensory properties of biscuit samples were evaluated by 10 panelists. The panelists were semi-trained to evaluate and compare the samples for parameters of appearance, color, smell, taste texture / mouth feel and overall acceptance, on a 9-point hedonic scale ranging from 1 for “dislike extremely” to “like extremely”, according to a modified method (*31*). In the preparatory session, the panel members had a thorough discussion in order to clarify each attribute before their evaluation. For each parameter, the average of the relevant scores given by the panelists was calculated.

Changes detected during the storage of biscuits

All biscuit samples were stored at room temperature (around 22-23 ºC) in locked sterile bags for a period of 45 days. The samples were measured for moisture content, peroxide value (PV), total acidity, mold-yeast count (ISO Method 21527-2) (*32*) and their pH values were measured on the 1st , 20th and 45th days. Fatty acid compositions of the biscuit samples were evaluated in the freshly baked samples and the samples that had been stored for 6 months, with gas chromatography GC – FID (Gas Chromatography- Flame Ionization Detector) model CLARUS 580 (Perkin Elmer, Shelton, CT, USA) and a BP x 70 column (30 m, 0.25 mm, 0.25 mm film thickness) (SGE Analytical Science, Australia). The fatty acid contents are given as their percentage of total oil content.

Statistical analysis

The data were analyzed using the SPSS software (version 18, SPSS Inc. Chicago) (*33*). Analysis of variance (ANOVA) was used for comparison. The Duncan test was used as the post-hoc test to determine the differences. Differences between samples were calculated at 95 % significance level (*34*).

RESULTS AND DISCUSSION

Nutritional evaluation

Sustaining the nutritional properties of gluten-free products are among the most recent focus of interest for the researchers, as traditional gluten-free ingredients/flours are low in proteins and antioxidants but only rich in carbohydrates (*8*).

Proximate analyses of raw materials

According to the current findings (Table 2), raw materials had significant differences (p<0.05) in terms of proximate components.

Buckwheat flour had significantly higher amount of protein (11.4 %) than the chestnut flour and potato flour, but lower than that of the chia seeds (p<0.05). Ash and fat contents and also the amount of carbohydrates (72.4 %) in buckwheat flour were not significantly different than the other flour sources (p>0.05). Similar to our findings, the moisture, protein, ash and fat contents of buckwheat flour that was used as the raw material for cookies with rice flour; were specified as 11.3 %, 12.3 %, 2.2 % and 2.9 %, respectively in a different study (*35*). According to another study, buckwheat flour carbohydrate content has been reported as 69.7 % (*36*).

Chestnut flour had significantly lower (p<0.05) protein content (5.2 %) than the other ingredients. This finding commensurates with previous findings reported in the literature, since chestnut flour was reported by Šoronja-Simović *et al.* (2017) and Lopes *et al.* (2016) to contain 5.3 % and 5.7 % protein, respectively (*37,38*). Its fat and carbohydrate contents were statistically different from buckwheat flour (p>0.05). As expected, buckwheat flour and chestnut flour also had higher amounts of total carbohydrates in comparison to that of chia seeds (p<0.05). Chestnut flour was depicted as having a lower moisture (7.7 %) and ash (2.1 %); but similar protein (5.7 %), fat (1.6 %), dietary fiber (20.7 %) and carbohydrate (62.3 %) contents, in earlier studies (*38*).

Total amounts of protein (6.7 %), ash (2.8 %) and carbohydrates (81.8 %) in potato flour were slightly different with respect to the other flour sources; although the fat content was significantly higher than the other ingredients (except for the chia seeds) and the moisture content was lower (p<0.05). In a recent study in the literature, potato flour was also used as a raw material for another gluten-free product and reported to contain 9.17 % protein, 3.86 % ash, 0.26 % fat and 20.51 % dietary fiber. Therefore, proximate contents found were generally higher than the potato flour used in this study (*11*). In contrast, the potato flour used in another study was reported to have an ash content of 2.90 %, protein 5.47 %, total carbohydrate 79.83 % and dietary fiber 5.29 %, thus being more similar to the raw material presented in this study (*39*).

Chia seeds had significantly higher dietary fiber (34.4 %), protein (23.1 %) and ash (4.58 %) but lower carbohydrates (36.2 %) in comparison to the flour sources (p<0.05) used in this study.

Comparable to the current findings, chia seeds were reported to have 9.3 % moisture, 25.1 % protein, 26.2 % fat and 5.5 % ash (*40*). Total dietary fiber was indicated as quite similar (around 37.4 %) (*41*), or slightly lower (29.6 % and 30.1 %) for chia seeds and chia seed flour, respectively (*42*) in comparison to our findings (34.4 %).

Proximate analyses of biscuit samples

According to the results of the proximate component analyses of biscuit samples (Table 3), the compositional differences in raw materials affected the proximate compositions of the biscuits, as expected ($p<0.05$).

Protein contents of the different biscuit samples were significantly different ($p<0.05$), the biscuits baked with chestnut flour having the lowest amount of protein (4.8 % d.b.), which is in accordance with the relatively lower protein content of the chestnut flour. Biscuits with buckwheat and chia had the highest amount of proteins (8.2 %, d.b.). Buckwheat flour had significantly higher amounts of protein (11.4 %) with respect to the chestnut and potato flours, although the level was lower than that of the chia seeds. Therefore, incorporation of buckwheat flour in gluten-free formulations was found to be an effective method for increasing the protein content in buckwheat containing biscuits. Similar results have been reported in a study using wholegrain buckwheat flour in gluten-free crackers (*43*). The increase in total protein content with chia seed flour was also similar to other studies, as the changing contents of chia seed flour from 5 to 20 % in the study by Kaur *et al.* (2015) resulted in increasing amounts of measured protein in biscuit samples, ranging from 9.7 to 11.7 % (*36*).

No significant differences were detected in the total ash and moisture contents of the different biscuit samples ($p>0.05$).

On the other hand, the fat contents of the biscuits were mainly affected by the addition of chia seeds, and biscuits with chia seeds had significantly higher levels of fat contents ($p<0.05$). Increases in the total dietary fiber and the fat contents of chia seeds added to biscuit samples were also evident in previous studies in the literature (*42*). Previous findings on the nutritional evaluation of gluten-free products that combine chia seeds and buckwheat flour highlighted that these products had health promoting benefits for obese and diabetic individuals by lowering their glycemic index, increasing satiety, in addition to being gluten-free (*44*).

Among all biscuit samples, the dietary fiber content was lower in biscuits with buckwheat ($p<0.05$). This finding was in parallel to the lower amount of dietary fiber in buckwheat flour.

Total phenolics in biscuit samples

The use of ingredients having relatively higher amount of phenolic compounds in gluten-free products has a significant benefit for celiac patients due to their ability to bind to fiber moieties, thus making them readily absorbable in the colon. In addition to the direct antioxidant activity of phenolic compounds, this binding ability was proposed as a beneficial mechanism for celiac patients by modifying the composition and immune function of the gut microbiota (*45*).

The total phenolic content of the biscuit samples changed between 248.2 and 400.2 mg GAE/100 g dry sample. Biscuits with chestnut had the highest phenolic content among all samples (400.2 mg GAE/100 g dry sample). Biscuits with buckwheat also contained relatively higher amounts of phenolics (387.0 mg GAE/100 g dry sample). In a previous review on chestnuts, high but varying contents of total phenolics (mainly as gallic and ellagic acids) in chestnut fruits (280–910 mg GAE/100 g and 610–2560 mg GAE/100 g) were mentioned (*46*).

Chia seed supplementation to the biscuit formulation, on the other hand, significantly decreased the total phenolics ($p<0.05$). The level of chia seed incorporation may be affective on the total phenolic contents. According to a previous research, total phenolic content was measured as 156.99 µg/g in cooked durum wheat pasta; while being lower in 5 % chia seed incorporated samples (123.53 µg/g), it was higher in 10 % chia seed incorporated samples (186.80 µg/g) (*8*). In addition, the relative decrease in total phenolic content after chia seed supplementation may be related with the richness of chia seeds not only with the nutritional compounds but also the non-nutritional healthy compounds (*44*).

Total antioxidant activity (TAA) in biscuit samples

TAA of the biscuits varied between 78.2 and 155.5 mg TE/100 g dry sample. In parallel with total phenolics, biscuits with chestnut had the highest TAA and the addition of chia seeds significantly reduced the measured TAA. In the literature, increases in the measured level of antioxidant activities after the baking of buckwheat flour and chestnut flour incorporated samples were also observed and this was explained as the effect of Maillard reaction products formed during heat treatment (*2,47*). Relatively lower antioxidant activity of chia seeds was also reported and related to the presence of hydrophilic phenolic acids in chia seeds (*48*).

Biscuit quality evaluation

Consumers mainly expect alluring quality characteristics such as a tender texture, good integrity and appealing flavor in biscuits/cookies.

Physical properties

The quality parameters of gluten-free biscuits are depicted in Table 3. Although, no significant differences were detected in the physical parameters of diameter, height, and mass of the biscuit samples ($p>0.05$), chia reduced the volumes of the biscuits slightly. This decreasing tendency in specific volumes was also evident in bread samples with chia seeds and in a previous study, protein network interruption was presented as the main reason for this change (*49*). However, the gluten network development in biscuits is quite limited because of its high fat and sugar contents. Therefore, the disruption of gluten network is not as critical in biscuits as it is in bread, and the effect of chia seeds on volume is rather restricted in the samples presented in this study (*9*).

Spread ratio (ratio of diameter/thickness) is among the most important characteristics for biscuits in determining their quality. Spread ratio values were higher in buckwheat blended biscuits in comparison to its chestnut blended counterparts ($p>0.05$). Moreover, chia seed seemed to cause a slightly increasing effect on spread ratio. In the previous studies, the spread ratio was strongly linked with the chemical composition of the raw materials in addition to the interference of sugar and fat and relatively lower dough viscosity (*14*).

Hardness

Hardness of biscuits is among the distinctive textural parameters for consumers and general textural properties of biscuits are highly related with the starch gelatinization and sugar, rather than the protein/starch structure (*9*). Hardness ranged between 30.1 and 48.7 N, with the difference between buckwheat and the remaining samples being statistically significant ($p<0.05$). Effect of buckwheat flour on the increased hardness of bakery products was also presented in the literature (*13, 31*). Buckwheat flour substitution was reported to increase the hardness (from 24.63 N to 42.30 N) in gluten free biscuits, in comparison to the wheat flour samples (*36*). The hardness was also presented to be undesirable during the shelf-life of buckwheat incorporated gluten free biscuits (*14*). Previously, presence of sucrose in chestnut flour was mentioned as a factor that affect the rheological properties of bakery products by inhibiting the hydration of starch granules and starch gelatinization (*37*). However, its effect was not as precise as the hardness of buckwheat samples. Decrease in the hardness with added chia seeds might be related with the ability of chia seeds to absorb more water (*36*).

According to the results, the highest water absorption was measured in the biscuits with buckwheat and chia (3.3 g). Use of chia seeds with either buckwheat or chestnut flour increased the measured amount of water absorption. Increase in water absorption of buckwheat flour and gum substituted gluten-free biscuits was delineated in the literature (*36*). The extent of water absorption is related to the interaction of water and the ingredients, the number of hydration positions and protein configuration. In this context, the presence of more protein and fiber in chia seed gel was suggested as the origin of the relatively higher amount of water (*50*) .

Color

Biscuit color is mostly related with the color of the ingredients, in addition to the browning during the advanced stage of the Maillard reaction and caramelization. Color parameters of darkness/lightness (*L** values), redness (*a** values), and yellowness (*b** values) in addition to total color change (ΔE) were determined and results are given in Fig. 1. Samples with buckwheat flour were lighter in color, having significantly higher *L** values in comparison to biscuits with chestnut flour ($p<0.05$). Addition of chia seeds seemed to make no significant changes ($p>0.05$) on the lightness. In chestnut substituted biscuits, redness values were slightly higher (higher a* values), while addition of chia seeds was a lowering factor for redness. Although yellowness was slightly higher in samples with buckwheat, the difference was not statistically significant ($p>0.05$). ΔE data revealed that, chestnut flour had a more distinct effect on the color of biscuits ($p<0.05$). In parallel to our findings, the use of chestnut flour as a substitute for wheat flour (increasing levels from 20 to 60 % and 0 to 100 %), provided the similar increasing effect on the redness of the cookies and bread samples, respectively (*37,51*). In the literature, chestnut flour with its relatively high content of sugar (20-32 %) and starch (50-60 %), was related with the caramelization and Maillard reactions due to baking (*2*). These reactions were characterized by the decreased *L** values in the samples, while increasing a* values were measured.

Sensory evaluation of the biscuits

The results of the sensory evaluation are presented in Fig. 2. According to the sensory evaluation results, only “texture” was indicated as significantly different among the biscuit samples, as the addition of chia seeds significantly increased the scores for the texture ($p<0.05$). The overall evaluation of the panelists illustrated a moderately higher acceptance ($p>0.05$) for biscuits supplemented with chia seeds (both buckwheat and chestnut flour based biscuits) and the biscuits formulated with buckwheat flour and chia had the highest scores for appearance, flavor and texture. Biscuits with buckwheat generally had the lowest results (except for the color and appearance) in terms of the sensory parameters evaluated. Studies indicating that the level of buckwheat flour substitution in biscuits affected their sensorial acceptance could be found in the literature (*52*), as 40

% substituted biscuits had the highest score among three levels (30, 40 and 50 %) of trials, while the overall acceptability was measured in the xanthan gum added samples. The presence of significant phenolic compounds such rutin, quercetin and protocatechuic acid in buckwheat flour was represented as the main factor for lower scores of buckwheat incorporated products (*52*).

In the literature, lower scores in sensory evaluation of buckwheat incorporated gluten-free biscuits were improved by adding gum acacia and guar gum into the formulations. Increase in consumer preferences were explained by different chemical interactions occurring between the hydrocolloids and other food ingredients. Comparable increasing inclinations after the addition of chia seeds into chestnut biscuits were also evident for flavor and overall acceptance results. This finding is similar to previous findings as combinations of chestnut flour and rice flour with different gums and emulsifiers provided increased acceptance scores in sensory evaluation of the bread samples (*15*).

Visual properties of appearance and color of biscuits with chestnut flour were ranked higher than the biscuits with buckwheat flour. Positive contribution of chestnut flour to the properties such as appearance, shape, crumb structure *etc*. was in parallel to the previous studies in the literature (*37*). More specifically, consumer preference for darker color in gluten-free bakery products was highlighted in previous studies (*19*).

Although the flavor scores were not significantly different among different formulations ($p>0.05$), chia seeds seemed to positively affect the flavor of buckwheat biscuits. Due to the perceived bitterness, the buckwheat flour was reported to affect the product flavor adversely particularly at high substitution levels (40 %) in the literature (*10*).

Changes detected during the storage of biscuits

Microbiological changes

No mold and yeast growth was detected on the 1st, 20th and 45th days. Changes in the moisture content during the storage of bakery products is a significant parameter for evaluation of their quality.

Physicochemical changes

According to the present results, no statistically significant differences were evident among the initial moisture contents of the biscuit samples (as of day 1) ($p>0.05$). Initial moisture contents measured in this study ranged between 5.2 % (the lowest for the biscuits with buckwheat) and 5.9 % (the highest for the biscuits with chestnut). During the considered storage period, all the samples tended to absorb moisture and significant increases were evident as of the 20th day of storage ($p<0.05$), for each sample (Table 4). On the other hand, a milder increase was evident after the 20th

day, as no more significant changes were measured from the 20th day to the 45th day of storage ($p>0.05$). During the storage period, the biscuits with chestnut absorbed more moisture in comparison to the biscuits with buckwheat ($p<0.05$). This finding was in accordance with the literature (*2*), indicating that chestnut containing biscuit samples had increased moisture content as the storage period progressed, particularly significant rises in moisture absorption were detected on the 30th and 60th days of the storage periods. Chia seeds had no effect on the moisture content of the biscuits.

Change in pH level during storage has been indicated as it might affect the perception of flavor in biscuits and values near neutral pH are more preferred. According to the results, pH levels of the samples changed between 4.96 and 5.62 as of day 1. Samples with chia seeds had significantly lower pH ($p<0.05$). Changes of pH during the storage period differed significantly for each sample. pH values decreased significantly ($p<0.05$) in biscuits with buckwheat (5.55 to 5.27) and biscuits with chestnut (5.62 to 5.55) during storage, and this was in accordance with the previous findings, as the incorporation of barley, gram, millet and maize flours similarly diminished the pH of the biscuits during ambient storage for 60 days (*53*). However, in contrast, the pH levels of both biscuits with chia seeds fluctuated during the storage period, although the only significant increase was evident for the biscuits with chestnut and chia from day 1 to 20. These findings were different from that of Mesias *et al.* (2016) who found that chia seed addition (5-20 %) into wheat flour resulted in a decreasing trend in pH levels from 7.6 to 7.3, in their research (*42*).

Peroxide value (PV)

PV is the most common indicator for measuring the oxidation during storage. Significant increases in PV values were detected for all samples ($p<0.05$). Oxidation stability of biscuits with chestnut and chia seeds was found to be lower than the remaining samples, at the end of the storage period, with relatively high PV (1.95 mmol O_2/kg). This is expected, since the total amount of chia seeds in the biscuit formulation is around 10 % of the total flour content in the biscuits with chia seeds, with mostly unsaturated fatty acids. In the literature, the PV of chia seed (10 %) added cookies was reported as 2.81 mmol O_2/kg at the end of 30 days of storage (*54*).

Fatty acid composition

The fatty acid compositions of the biscuit samples (after the 1st day of storage and after the 6th month of storage) are presented in Table 5. Initially, only the caprylic acid, palmitic acid and margaric acid differed significantly among different biscuits ($p<0.05$). Palmitic, oleic, linoleic, lauric and stearic acids are the dominant fatty acids in the biscuit samples produced.

Throughout the shelf life, the losses in lauric, stearic and α-linolenic acids were evident ($p<0.05$). Other biscuit samples were relatively stable during the period they were stored. The use of margarine in the biscuit formulations and the presence of antioxidants from buckwheat and chestnut flours might have contributed to this stability (*2*). Chia seeds have a high amount of total fat, mostly rich in α-linolenic acid (ω3) and also linoleic acid (ω6). In the literature, different colors of chia seeds were detected with no difference in their fatty acid profiles (*55*). According to the findings, palmitic, stearic, oleic, linoleic and α-linolenic fatty acids were detected in both white and black-spotted chia seeds from five different locations in addition to trace amounts of myristic, arachidic, gadoleic, behenic, eracic, and lignoceric acids. However, the total amount of oil in chia seeds and palmitic, oleic, linoleic and α-linolenic fatty acids among oils from different locations were significantly different (*55*). Therefore, the relatively lower amount of α-linolenic acid (ω3) might be related with the type of chia seeds used in the formulations.

CONCLUSIONS

Recent research on gluten free products led to the improvement of nutritional profile of these products as the primary point of interest. The research presented here confirms the importance of using functional ingredients in gluten-free product formulations as well as monitoring the storage stability. The results demonstrated that due to their relative stability during storage, particularly the biscuits containing chestnut flour and chia seeds have the potential to be used in different gluten-free product formulations (in biscuits, crackers, snacks *etc.*) in the future with respect to their significant protein and dietary fiber contents, superior product quality and better sensory acceptability.

CONFLICTS OF INTEREST

Authors state no conflict of interests with respect to the objective, interpretation, and presentation of the results in this study.

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AUTHORS' CONTRIBUTIONS

In this study, the experiments, formal analysis and writing of the original draft were implemented by G. Silav Tuzlu; the conceptualization, methodology development, supervision and reviewing and editing were implemented by Z. Tacer Caba.

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Table 1. Biscuit formulations

Ingredients	Biscuits with buckwheat	Biscuits with chestnut flour	Biscuits with buckwheat and chia seeds	Biscuits with chestnut and chia seeds
m (buckwheat flour)/g	75	-	75	-
m (chestnut flour)/g	-	75	-	75
m (potato flour)/g	75	75	75	75
m (chia seeds)/g	-	-	15	15
m (sugar)/g	55	55	55	55
V (lactose free milk)/mL	40	40	45	45
m (margarine)/g	45	45	45	45
V (eggs)/mL	6	6	6	6
m (sodium bicarbonate)/g	3	3	3	3

Table 2. Proximate analyses in raw materials

Raw materials	*w* (protein)/%	*w* (dietary fiber)/%	*w* (fat)/%	*w* (ash)/%	*w* (CHO)/%	*w* (moisture)/%	*E* (kCal/100g)
Buckwheat flour	(11.4±0.2)^b	(7.6±0.3)^c	(1.7±0.1)^b	(1.88±0.15)^b	(72.4±0.3)^a	(12.9±0.1)^b	(320.0±0.3)^b
Potato flour	(6.7±0.2)^c	(6.4±0.5)^c	(3.0±0.1)^{ab}	(2.80±0.2)^{ab}	(81.1±0.4)^a	(6.4±0.2)^c	(353.1±0.5)^{ab}
Chestnut flour	(5.2±0.1)^c	(18.0±0.2)^b	(1.6±0.0)^b	(3.83±0.09)^a	(68.4±0.2)^a	(21.0±0.1)^a	(236.5±0.3)^c
Chia Seed	(23.1 ±0.1)^a	(34.4±0.4)^a	(29.3±0.0)^a	(4.58±0.04)^a	(36.2±0.1)^b	(6.8±0.1)^c	(363.3±0.2)^a

The mean value ± standard deviation of duplicate analyses are given in the table. Values in the same column and different letters (a-c) differ significantly ($p<0.05$).
E: Total energy, CHO: Carbohydrates

Table 3. Proximate components, total phenolics, antioxidant activity and quality parameters of biscuits

Sample	*w* (protein)/ %	*w* (ash)/ %	*w* (moisture)/ %	w (fat)/ %	*w* (dietary fiber)/ %	*E* (kCal/ 100g)	Total phenolics	*TAA*	d/ (mm)	*h*/ (mm)	*m*/ (g)	*SV*/ (cm^3/ g)	Spread ratio	*WA*/(g)	Hardness/ (N)
Biscuit with buckwheat	(7.1±0.0)^{b}	(3.7±0.4)^{a}	(5.2±0.3)^{a}	(17.6±0.0)^{b}	(11.5±0.0)^{d}	(406.7±0.3)^{a}	(387.0±9.1)^{a}	(132.2±5.9)^{b}	(60.0±0.9)^{a}	(11.1±0.4)^{a}	(20.2±1.3)^{a}	(1.25±0.03)^{a}	(5.4±0.0)^{a}	(2.6±0.0)^{b}	(48.7±3.7)^{a}
Biscuit with chestnut	(4.8±0.4)^{d}	(3.7±0.6)^{a}	(5.9±0.3)^{a}	(17.7±0.0)^{b}	(13.2±0.0)^{c}	(397.5±2.3)^{ab}	(400.2±3.7)^{a}	(155.5±4.4)^{a}	(58.1±2.7)^{a}	(11.4±0.8)^{a}	(20.8±2.9)^{a}	(1.24±0.22)^{a}	(5.1±0.1)^{a}	(2.5±0.1)^{b}	(38.3±5.7)^{b}
Biscuit with buckwheat and chia	(8.2±0.1)^{a}	(3.7±0.6)^{a}	(5.4±0.8)^{a}	(18.8±0.1)^{a}	(17.2±0.1)^{b}	(388.6±0.5)^{b}	(248.2±7.9)^{c}	(78.2±2.3)^{d}	(60.2±1.3)^{a}	(10.7±1.1)^{a}	(22.0±2.3)^{a}	(1.14±0.01)^{b}	(5.6±0.7)^{a}	(3.3±0.2)^{a}	(37.6±8.1)^{b}
Biscuit with chestnut and chia	(5.7±0.2)^{c}	(3.8±0.6)^{a}	(5.5±0.5)^{a}	(18.3±0.4)^{ab}	(19.3±0.1)^{a}	(376.8±4.2)^{c}	(298.5±3.3)^{b}	(112.4±0.2)^{c}	(59.3±1.2)^{a}	(11.3±0.5)^{a}	(21.9±3.0)^{a}	(1.16±0.17)^{b}	(5.3±0.2)^{a}	(3.2±0.0)^{a}	(30.1±0.1)^{b}

Antioxidant activity is given in mg TE/ 100 g dry sample. Total phenolics are given in mg GAE/100 g dry sample. The mean value ± standard deviation of duplicate analyses are given in the table. Values in the same column and different letters (a-d) differ significantly ($p<0.05$). Values in the same column and different letters differ significantly ($p<0.05$). Diameter: d, Height: h; Mass: m; SV: Specific Volume; WA: Water Absorption

Table 4. Changes during the storage of biscuits

Sample	Day 1			Day 20			Day 45		
	w (moisture)/ %	pH	*PV*/ (mmol O_2/kg)	*w* (moisture)/ %	pH	*PV*/ (mmol O_2/kg)	*w* (moisture)/ %	pH	*PV*/ (mmol O_2/kg)
Biscuit with buckwheat	(5.2±0.3)^{ay}	(5.57±0.01)^{ax}	(1.08±0.01)^{cy}	(8.3±1.0)^{bx}	(5.40±0.00)^{by}	(1.14±0.01)^{cy}	(8.8±0.3)^{bx}	(5.27±0.01)^{bz}	(1.31±0.11)^{cx}
Biscuit with chestnut	(5.9±0.3)^{ay}	(5.62±0.02)^{ax}	(1.55±0.04)^{ay}	(11.0±1.0)^{ax}	(5.60±0.01)^{ax}	(1.72±0.16)^{ax}	(11.8±0.2)^{ax}	(5.55±0.01)^{ay}	(1.81±0.03)^{bx}
Biscuit with buckwheat and chia	(5.4±0.8)^{ay}	(5.18±0.03)^{bx}	(1.11±0.05)^{cy}	8.9 ± 0.5^{bax}	(5.17±0.01)^{dx}	(1.22±0.04)^{cy}	(9.1±0.4)^{bx}	(5.21±0.01)^{cx}	(1.42±0.16)^{cx}
Biscuit with chestnut and chia	(5.5±0.5)^{ay}	(4.96±0.04)^{cy}	(1.30±0.06)^{bz}	10.7±0.4^{ax}	(5.23±0.01)^{cx}	(1.50±0.06)^{by}	(11.2±0.7)^{ax}	(5.28±0.01)^{bx}	(1.95±0.03)^{ax}

The mean value ± standard deviation of duplicate analyses are given in the table. Among each of moisture (%), pH and peroxide value (PV); values with different letters (a-c) within the same column and different letters (x-z) within the same row differ significantly ($p<0.05$).

Table 5. Fatty acid compositions of the biscuit samples (%)

Samples	Biscuit with buckwheat	Biscuit with chestnut	Biscuit with buckwheat and chia	Biscuit with chestnut and chia	Biscuit with buckwheat	Biscuit with chestnut	Biscuit with buckwheat and chia	Biscuit with chestnut and chia
	1st Day of Storage				After 6th Month of Storage			
(C4:0) Butyric acid	(0.24±0.01)^{A}	(0.11±0.02)^{A}	(0.11±0.02)^{A}	(0.24±0.09)^{A}	(0.18±0.08)^{a} -	(0.28±0.13)^{a} -	(0.17±0.04)^{a} -	(0.27±0.13)^{a} -
(C8:0) Caprylic acid	(2.72±0.04)^{A}	(1.27±0.08)^{B}	(1.62±0.18)^{B}	(3.03±0.47)^{A}	(1.50±0.64)^{a} -	(1.71±0.79)^{a} -	(1.29±0.16)^{a} *D	(2.01±0.91)^{a} -
(C10:0) Capric acid	(1.66±0.21)^{A}	(1.11±0.00)^{A}	(1.32±0.10)^{A}	(2.02±0.62)^{A}	(1.13±0.4)^{a} -	(1.32±0.63)^{a} -	(0.97±0.06)^{a} -	(1.42±0.68)^{a} -
(C12:0) Lauric acid	(14.33±2.34)^{A}	(12.72±0.16)^{A}	(13.58±1.33)^{A}	(17.53±5.20)^{A}	(9.81±2.08)^{a} *D	(10.88±4.89)^{a} -	(8.91±2.32)^{a} -	(11.02±5.66)^{a} -
(C14:0) Myristic acid	(4.21±0.29)^{A}	(4.44±0.10)^{A}	(4.24±0.52)^{A}	(4.67±0.95)^{A}	(3.49±0.16)^{a} -	(3.84±0.98)^{a} -	(3.38±0.81)^{a} -	(3.78±1.75)^{a} -
(C16:0) Palmitic acid	(28.7±0.74)^{AB}	(30.31±0.40)^{A}	(29.19±1.22)^{AB}	(27.78±0.07)^{B}	(31.45±1.52)^{a} -	(29.34±0.25)^{a} -	(29.58±1.69)^{a} -	(29.84±1.01)^{a} -
(C16:1) Palmitoleic	(0.24±0.00)^{A}	(0.29±0.01)^{A}	(0.30±0.08)^{A}	(0.26±0.01)^{A}	(0.23±0.02)^{a} -	(0.19±0.00)^{a} *D	(0.17±0.01)^{a} -	(0.29±0.10)^{a} -
(C17:0) Margaric acid	(0.08±0.00)^{B}	(0.09±0.00)^{B}	(0.08±0.00)^{B}	(0.44±0.05)^{A}	(0.08±0.01)^{a} -	(0.09±0.01)^{a} -	(0.08±0.01)^{a} -	(0.10±0.03)^{a} *D
(C18:0) Stearic acid	(10.25±0.43)^{A}	(9.98±0.21)^{A}	(10.68±0.88)^{A}	(9.00±1.76)^{A}	(7.14±0.25)^{a} *D	(7.89±1.12)^{a} -	(8.19±0.78)^{a} -	(7.97±0.22)^{a} -
(C18:1) Elaidic acid	(0.09±0.00)^{A}	(0.10±0.00)^{A}	(0.13±0.02)^{A}	(0.10±0.02)^{A}	(0.07±0.01)^{a} -	(0.12±0.04)^{a} -	(0.10±0.01)^{a} -	nd -
(C18:1) Oleic acid	(20.96±0.95)^{A}	(21.75±0.33)^{A}	(21.60±1.46)^{A}	(19.48±3.32)^{A}	(28.11±0.97)^{a} *I	(31.14±4.21)^{b} -	(32.45±2.66)^{a} -	(29.49±0.00)^{a} -
(C18:2) Linoleic acid (ω6)	(14.67±0.64)^{A}	(15.73±0.23)^{A}	(15.16±0.98)^{A}	(16.09±2.34)^{A}	(15.19±0.57)^{b} -	(11.29±1.48)^{c} -	(12.42±0.98)^{bc} -	(19.69±1.37)^{a} -
(C18:3) α-Linolenic acid (ω3)	(1.31±0.11)^{A}	(1.50±0.02)^{A}	(1.53±0.13)^{A}	(1.48±0.26)^{A}	(0.73±0.06)^{b} *D	(0.99±0.18)^{ab} -	(1.25±0.10)^{a} -	(1.06±0.07)^{a} -
(C20:0) Arachidic acid	(0.31±0.02)^{A}	(0.29±0.00)^{A}	(0.35±0.02)^{A}	(0.27±0.10)^{A}	(0.38±0.01)^{a} *I	(0.40±0.08)^{a} -	(0.43±0.07)^{a} -	(0.36±0.06)^{a} -

[1]The mean value ± standard deviation of duplicate analyses are given in the table. Different letters in the same rows for the 1st day of storage (A-B) and 6th month of storage (a-c) differ significantly ($p<0.05$). When the difference between the 1st day of storage and 6th month of storage was insignificant ($p>0.05$): **-** , if significantly increased ($p<0.05$): ***I** and if significantly decreased ($p<0.05$):

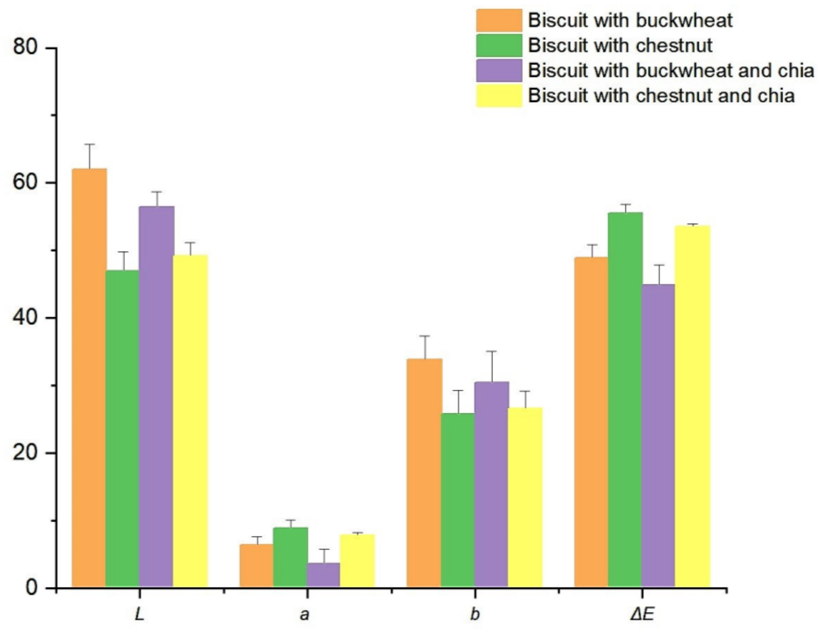

Fig. 1. Color measurements of biscuit samples

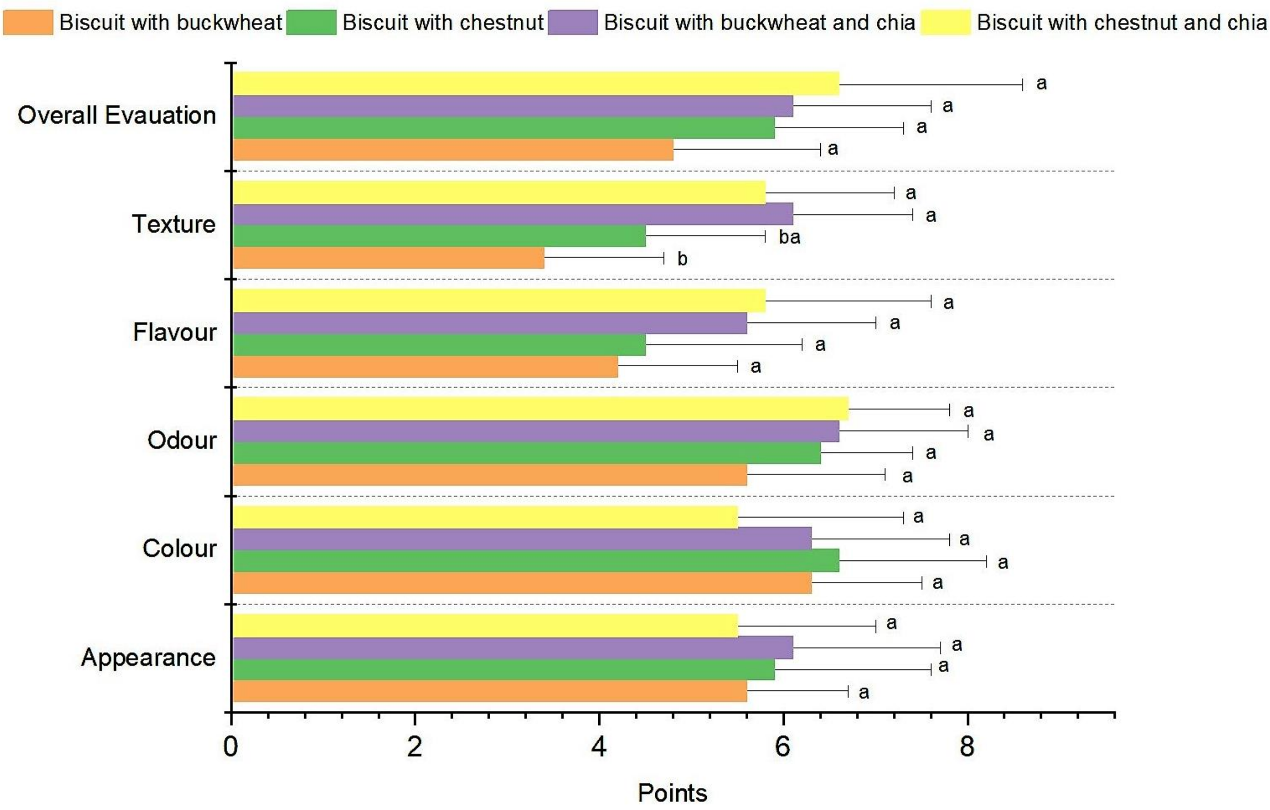

Fig. 2. Sensory evaluation of the samples. The mean value ± standard deviation of sensory analyses are given in the figure. Different letters among each parameter column, differ significantly ($p<0.05$)