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RESEARCH PAPER

Formulation of omega-3 rich walnut (*Juglans regia* L.) Biscuits and its nutritional facts, organoleptic evaluation and antioxidant activities

Muhammad Khalid Saeed[1]*, Naseem Zahra[1], Shafaq Ayman[2], Falak Shair[2], Uzair Bin Aamir[3], Saad Ahmed Khan[3] and Mateen Abbas[4]

[1]Food and Biotechnology Research Centre, PCSIR Laboratories Complex, Lahore, Pakistan
[2]Department of Botany, Government College University, Lahore, Pakistan
[3]Dr. Ikram-ul-Haq Institute of Industrial Biotechnology, GC University, Lahore Pakistan
[4]Quality Operations Laboratory, Institute of Microbiology, University of Veterinary and Animal Sciences 54000, Lahore, Pakistan

*Corresponding author's email: rosefbrc1@gmail.com

Key Message: This study indicates that by adding the walnut powder in the formulation of biscuits enhanced the nutritional value especially omega-3 fat as well as high value of polyphenols and antioxidant properties. From this study it is also narrated that 7.5% walnut powder formulated biscuits was acceptable that may be used for formulation of various value added food products.

Abstract

The need for healthy biscuits is rising these days. A healthy diet is positively correlated with the inclusion of bioactive components and the reduction of sugar. Omega-3 fatty acids and other naturally occurring bioactive substances found in walnuts (*Juglans regia* L.) may have a significant impact on the treatment of chronic diseases and other degenerative conditions. The current study investigated the effect of walnut powder at different levels of substitution (2.5, 5, 7.5 & 10%) in the formulation of biscuits on the nutritional facts, sensory evaluation, polyphenols and antioxidant potentials. The total polyphenolic content of formulated biscuits as well as control biscuits with 100% of wheat flour were assessed by Folin-Ciocalteu reagent and its antioxidant activity was deliberated by 2,2-diphenyl-1-picryl-hydrazyl-hydrate (DPPH) assay, while sensory evaluation was assessed by hedonic scale. The results showed that an increase in walnut powder resulted in a significant ($p<0.05$) increase in omega fat ranging from (12.18±0.10–22.49±0.53 g/100 g), dietary fiber (2.5±0.01–4.4±0.04 g/100g) and protein (13.41±0.02 –15.88±0.14 g/100 g). The formulate biscuits' total phenolic content ranged from 30.21–47.7 mg GAE/g, and their DPPH radical scavenging activity (% Inhibition) varied from 32.5±0.67–72.2±1.55 at 2–10 mg/ mL concentration. The sensory evaluation results revealed that the addition of 7.5% walnut powder resulted in favorable color, taste, aroma, and acceptability of the biscuits. However, increasing the walnut powder concentration to 10% resulted in a darker biscuit crust. From this study, it was concluded that by adding 7.5% walnut powder formulated biscuits have a good acceptability with natural antioxidants and rich in omega-3. Conclusively, walnut powder could therefore be utilized in developing biscuits as a functional and nutraceutical component. © 2026 The Author(s)

Keywords: Antioxidant activities, Nutritional facts, Omega-3 biscuits, Organoleptic evaluation, Sensory evaluation, Walnut

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Introduction

The relationship between dietary patterns and human health has received considerable scientific attention, particularly with increasing interest in the development of functional foods that provide health benefits beyond basic nutritional requirements (Fekete et al., 2025; Ma et al., 2026). Functional food products enriched with naturally occurring bioactive compounds are gaining popularity because of their potential to support human health and reduce the risk of chronic diseases (Zhang et al., 2022). Walnut (*Juglans regia* L.) is a deciduous broadleaf tree belonging to the family *Juglandaceae* and is widely cultivated worldwide. Walnuts are nutrient-rich nuts, containing abundant unsaturated fatty acids and bioactive compounds (Qin et al., 2026). For usage in the food, cosmetic, and pharmaceutical industries, the kernel, fresh green fruit, husk, shell, skin, bark, leaves, and root have all been extensively researched. Walnuts are particularly valuable due to their distinctive nutritional composition, including polyunsaturated fatty acids (PUFAs), especially omega-3 alpha-linolenic acid (ALA), dietary fibre, high-quality proteins, vitamins, minerals, and diverse phytochemicals (Rashki et al., 2024; Zhao et al., 2024; Al-Subaie & Shori, 2026). Regular walnut consumption has been associated with improved cardiovascular health, favourable lipid profiles, and reduced oxidative stress, making walnuts a promising ingredient for the formulation of nutritionally enhanced bakery products

(Fukasawa et al., 2024; Bereda et al., 2026). Walnut kernels contain several phenolic compounds, including phenolic acids, flavonoids, tannins, and other polyphenolic constituents, which contribute to their antioxidant potential (Cui et al., 2024; Qingyang et al., 2024). These compounds can neutralize reactive oxygen species, inhibit oxidative reactions, and help protect cellular components against oxidative damage. Recent research has also highlighted the antioxidant and anti-inflammatory properties of walnut-derived bioactive compounds, suggesting their potential role in modulating oxidative stress and inflammation (Rahbardar et al., 2025).

Because of its useful qualities, wheat flour is a key ingredient in the creation of all baked goods, including bread, doughnuts, biscuits, buns, and cakes. Developing nations struggle to grow enough wheat to meet domestic demand due to climate change and a lack of technology. As a result, they rely heavily on wheat imports, which have an impact on the nation's economy (Ojewumi et al., 2021). The use of flours derived from cassava, potatoes, breadfruit, plantains, and nuts has been encouraged for biscuit production and other food applications (Akuirene et al., 2026a). Because of its flavor, broad appeal, and nearly endless shelf life, biscuits have a long history and are consumed by people from all social strata. When produced from wheat flour, biscuits have remarkable baking qualities. However, they are unsuitable for regular dietary consumption since they are overly high in calories and carbohydrates but noticeably low in fiber, vitamins, and minerals (Abedin et al., 2026). Bakery products are a part of what humans need to eat. Biscuits are among the most widely consumed baked goods in the world because they are nearly ready-to-eat, have a long shelf life, and have reasonable price (Abedin et al., 2026).

Antioxidants are thought to protect living things against reactive oxygen species because they have the ability to scavenge free radicals (Manful et al., 2025). Even while the bodies of living things have their own antioxidant defense and repair systems, these systems are insufficient to counteract the harm that reactive oxygen species inflict. Consequently, dietary antioxidant supplements can support the maintenance of an adequate level of antioxidant status, which is promising for enhancing normal physiological function in all living things (Saeed et al., 2024). Vegetables, herbal plants, and foods rich in bioactive compounds are excellent sources of exogenous antioxidants. According to scientific investigations, synthetic antioxidant compounds, particularly tert-butylhydroquinone (TBHQ), butylated hydroxytoluene (BHT), and butylated hydroxyanisole (BHA), have been widely employed to prevent lipid peroxidation in food products. Research on nuts, fruits, vegetables, and other plant sources to find the natural antioxidants in them has been encouraged in recent years by the growing interest in finding substitutes for synthetic antioxidants from food components (Najjar et al., 2022). Around the world, biscuits are a popular cereal snack, especially for kids. These are usually little goods prepared from wheat flour that are either savory or sweet. Products made only of wheat flour should be supplemented with beneficial substances to make them healthier and strong (Yadav et al., 2022). In fact, biscuits are a common dietary item that is enjoyed by people of all ages, including children, adults, and the elderly.

The nutritional importance of walnuts is further attributed to their high content of ALA, an essential plant-derived omega-3 fatty acid that contributes to the maintenance of normal physiological functions. Recent meta-analytical evidence involving randomized controlled trials has demonstrated that walnut consumption can improve selected blood lipid parameters, particularly by reducing total cholesterol, LDL-cholesterol, and triglyceride concentrations (Mashayekhi et al., 2026). Furthermore, evidence from clinical studies suggests that walnut consumption may improve vascular endothelial function, as indicated by improvements in flow-mediated dilation (Hsu et al., 2024). Incorporating walnut kernels into biscuit formulations therefore represents a promising approach to improving the nutritional quality of conventional bakery products while enhancing their functional properties (Qin et al., 2026). The addition of walnut flour or ground walnut kernels may increase the protein, dietary fibre, unsaturated fatty acid, and phenolic contents of biscuits, although the extent of improvement depends on the incorporation level and processing conditions (Rabrenović et al., 2022). However, the substitution of wheat flour with walnut ingredients may also influence dough characteristics, biscuit texture, colour, flavour, and overall consumer acceptability (Qin et al., 2026). Therefore, optimizing walnut incorporation is essential to achieve an appropriate balance between nutritional enhancement and desirable sensory characteristics. Moreover, evaluating total phenolic content and antioxidant activity is important for determining whether the bioactive constituents of walnuts are retained following biscuit processing. Accordingly, the purpose of this study was to evaluate the impact of substituting a portion of wheat flour with omega-3 rich walnut powders on the nutritional value, antioxidant qualities, and polyphenols of biscuits as well as their consumer appeal.

Materials and Methods

Procurement of raw materials

Walnuts, wheat flour, canola oil, baking powder and refine sugar were procured from local market Lahore, Pakistan.

Development of biscuits

Additionally, the biscuit was prepared using flour devoid of plant materials as a control. The same flour was used to make biscuits with varying amounts of walnut powder. These biscuits were made with the usual ingredients (sugar, canola oil, baking powder, and water) together with high-protein flour and antioxidant-rich walnuts (Table 1). The components were quickly combined to make a uniform mixture. After that, water was added and stirred to make homogeneous solution. The

powdered walnuts were added all at once and blended at a medium speed. In the last stage, the dough was combined with baking powder and wheat flour. The dough was sheeted to a thickness of 4 mm, formed using a 4 cm diameter ring mold, and baked for 9 minutes at 150°C in an electric convection oven. In order to achieve a crispy texture, the biscuit samples were chilled for around ten minutes (Bakar et al., 2022). It is anticipated that these ingredients may enhance the biscuits' functional value and nutritional worth (Feumba et al., 2026). The following standard recipes were used in the FBRC, PCSIR Lahore laboratory to prepare the antioxidant-rich and high-protein biscuit samples, which were fortified with underutilized walnut powder:

Table 1 Ingredients used in different treatments for formulation of (omega-3) biscuits

Ingredients (g)	Control (T0)	Sample 1 (T1)	Sample 2 (T2)	Sample 3 (T3)	Sample 4 (T4)
Wheat flour	500	497.5	495	492.5	490
Walnut powder	-	2.5	5.0	7.5	10.0
Powder sugar	65	65	65	65	65
Canola oil	125	125	125	125	125
Baking powder	4	4	4	4	4
Water	200	200	200	200	200

Proximate composition

Moisture, ash, protein, fat, fiber, carbohydrates, and calories were measured in the biscuit samples. The Official Methods of Analysis of AOAC (2019) were used to determine the proximate analyses. Prior to analysis, samples were ground into a powder. Every sample was examined three times.

Sensory evaluation of biscuits

Ten members of semi-trained panels at the Food and Biotechnology Research Centre, PCSIR Lahore, formulated biscuits with varying concentrations of walnut powder with different numbers and submitted them for sensory evaluation. Using the control biscuit as the benchmark for assessment, the panelists were asked to grade each sensory characteristic. On a 9-point hedonic scale, which ranged from 9 (like highly) to 1 (dislike greatly), biscuits were assessed for appearance, surface colors, aroma, texture, taste and over all acceptability as described by Akuirene et al. (2026b).

Preparation of methanolic extract of biscuits

Using a slightly modified version of Castillo-López et al. (2017), the biscuits were extracted by maceration. In this study, 80% methanol was utilized as the solvent while biscuits were ground. Whatman filter paper No. 5 was used to filter the supernatant after centrifugation was carried out for 25 minutes at 4200 rpm. Before being examined further, each stock sample extract was kept at 4°C.

Total phenolic analysis

The Folin-Ciocalteu method, which is based on the reduction of the Folin-Ciocalteu reagent by phenolic component to create molybdenum-tungsten (which has a blue color) to be quantified spectrophotometrically, was used to assess the methanolic extract of the prepared biscuit for TPC. Singleton (1999) served as the basis for the total phenolic analysis, which was slightly modified by Jiao et al. (2026). Every sample extract was diluted to a 30 mg/mL concentration. The standard was gallic acid. An Ultraviolet-visible (UV-Vis) Spectrophotometer was used to detect the absorbance at 725 nm, and the TPC of biscuit extracts was expressed in gallic acid equivalent (GAE) (mg GAE/g).

Antioxidant activity determination

The DPPH method was used to assess the antioxidant activity of the biscuit's methanolic extract according to method given in literature (Brand-Williams et al., 1995). DPPH in methanol changed color, indicating the presence of antioxidants in tested samples which was initially dark purple to yellow color, when it reacted with the antioxidant, the hydrogen atom from the antioxidants attached itself to the radical form hydrazine, which decreased the conjugated double bonds in DPPH (Saeed et al., 2025). The extracts from functional biscuit samples (T0, T1, T2, T3, and T4) were diluted to produce a serial concentration that ranged from 2 mg/mL to 10 mg/mL. 2.9 ml DPPH solution and 0.1 ml of samples extract was taken in a test tube and incubated for 30 minutes in dark. The absorbance at 517 nm was measured using the UV-Vis Spectrophotometer. Antioxidant activity is expressed using the IC50 value, which shows the concentration of antioxidant (sample) to reduce 50% of free radical activity. A lower IC_{50} value indicates higher antioxidant activity.

Statistical analysis

Analyses were carried out twice, and all experiment values were expressed as the mean ± SD of two experiments. IBM SPSS Statistics version 25.0 (SPSS Inc., Chicago, Illinois, USA) was used to compare the values using one-way Analysis of Variance (ANOVA) with post hoc Duncan. $P < 0.05$ was used as the significant criterion.

Results

Table 2 shows the nutritional facts of walnut-rich biscuits in comparison to control biscuits. The findings demonstrated that

the addition of walnut-rich biscuit powder raised the amount of fat from 12.18±0.10 to 22.49±0.53%, the amount of protein from 13.41±0.02 to 15.88±0.14%, the amount of ash from 2.12±0.03 to 2.59±0.01%, and the amount of total dietary fiber from 2.5±0.01 to 4.4±0.04%. The percentage of carbohydrates range from 65.1±1.42 to 49.78±1.10%. In contrast, the energy increased from 423±3.90 to 465±4.12 Kcal/100g (T0–T4). There was a substantial difference between walnut powder 2.5% to walnut powder 10% in terms of total dietary fat. According to the study's findings, underutilized walnuts are a rich source of energy, fiber, protein, and omega-3 fat. The addition of walnut powder had a substantial impact on the nutrients of the biscuit samples ($p<0.05$).

Table 2 Nutritional facts of control and antioxidants walnuts rich (omega-3) biscuits

Parameters	% Values Control (T0)	% Values Sample-1 (T1)	% Values Sample-2 (T2)	% Values Sample-3 (T3)	% Values Sample-4 (T4)
Moisture	4.78±0.05	4.63±0.05	4.59±0.11	4.77±0.06	4.86±0.07
Ash	2.12±0.03	2.30±0.05	2.45±0.03	2.57±0.01	2.59±0.01
Fat	12.18±0.10	14.58±0.45	16.37±0.06	19.39±0.23	22.49±0.53
Crude fiber	2.5±0.01	3.10±0.03	3.70±0.04	4.20±0.04	4.4±0.04
Crude protein	13.41±0.02	14.83±0.05	14.86±0.12	14.97±0.14	15.88±0.14
Carbohydrates	65.1±1.42	64.56±1.31	58.03±1.25	54.1±1.11	49.78±1.10
Energy (Kcal/ 100 g)	423±3.90	433±3.96	439±3.99	451±4.05	465±4.12

Data are represented as ± SD

Sensory evaluations of biscuits formulated with different walnut powder levels

Table 3 displays the sensory measurements of biscuits made with varying amounts of walnut powder (2.5, 5.0, 7.5, and 10%). Sensory evaluation was done, including appearance, surface colors, aroma, texture, taste and over all acceptability. The information gathered showed that the biscuit samples supplemented with 7.5% walnut powder had the best results for every tested parameter. In terms of appearance, surface colors, aroma, texture, taste and over all acceptability, they were all extremely similar to the control formed samples, with levels of 7.38, 7.26, 7.53, 6.89, 7.63, and 7.32 compared to the controls, which were 7.30, 7.70, 7.90, 7.20, 7.50, and 7.70, respectively. However, as the doses of walnut powder supplementation increased, all the assessed metrics declined. In comparison to the 2, 5, and 7.5% additions, the 10% addition had the lowest values across all measurements (6.6, 6.8, 6.7, 6.5, 7.1 and 6.9 for appearance, colors, aroma, texture, taste and acceptability, respectively.

Table 3 Organoleptic evaluation of control and walnut-enriched (7.5%) formulated biscuits

Sr. No. of Judges	Appearance	Surface colour	Aroma	Texture	Taste	Acceptability
1	7.60±0.26	6.70±0.08	7.80±0.28	6.70±0.07	7.40±0.21	7.50±0.24
2	7.30±0.21	7.00±0.11	7.40±0.21	7.00±0.10	7.70±0.27	7. 40±0.22
3	7.40±0.22	7.80±0.29	6.50±0.06	6.80±0.08	7.60±0.11	7.60±0.28
4	7.50±0.24	7.30±0.21	7.70±0.27	7.30±0.20	7.90±0.28	7.20±0.181
5	7.20±0.19	7.50±0.24	7.40±0.20	6.40±0.05	7.40±0.10	7.10±0.12
6	7.50±0.25	6.90±0.09	7.60±0.26	7.10±0.13	7.90±0.19	7.00±0.11
7	7.40±0.21	7.10±0.12	7.70±0.28	6.60±0.06	7.40±0.12	7.50±0.23
8	7.60±0.27	7.50±0.16	7.40±0.21	7.00±0.11	7.80±0.27	7.70±0.29
9	7.30±0.20	6.60±0.07	7.30±0.21	6.80±0.08	7.70±0.20	6.90±0.09
10	7.00±0.10	7.20±0.19	7.50±0.24	7.20±0.19	7.50±0.23	7.30±0.20
Mean Score	7.38±0.21	7.26±0.19	7.53±0.25	6.89±0.10	7.63±0.25	7.32 ± 0.21

Data are represented as means ± SD

Total phenol content of biscuits

All of the treatments showed a significant ($P<0.05$) variation in total phenol content. The T0, T1, T2, T3, and T4 samples had total phenol concentrations of 35.7, 54.5, 85.8, 107.5, and 126.4 mg GAE/100g. When compared to control biscuits, functional biscuits made by combining walnut powder (7.5%) and (10%) had a considerably ($P<0.05$) higher total phenolic content (Fig. 1). The increase in total phenolic content may be attributed to the naturally high concentration of phenolic compounds in walnut powder. A gradual increase in walnut powder incorporation resulted in higher phenolic content, indicating its potential to enhance the antioxidant properties of biscuits.

Table 4 The antioxidant activity (IC_{50}) of (Omega-3) formulated biscuits

Sr. No.	Samples	IC_{50} Value (mg/mL)
1	T0 (Control) No walnuts	18.20 ± 1.07
2	T1 (walnuts powder 2.5%)	10.25 ± 0.85
3	T2 (walnuts powder 5.0 %)	7.26 ± 0.53
4	T3 (walnuts powder 7.5%)	5.21 ± 0.28
5	T3 (walnuts powder 10.0%)	3.51 ± 0.16

Data are represented as ±SD

Antioxidant activity of formulated Biscuits

These findings unequivocally indicate that the biscuit samples made with higher amounts of walnut powder (2.5–10%) exhibit higher levels of antioxidant activity. The current study also concludes that the introduction of walnut powder has improved the biscuits' antioxidant capacity.

Table 4 demonstrates that the IC_{50} value of formulated biscuits (T1-T4) with varying amounts of walnut powder ranged from 3.51±0.16 to 10.25±0.85 mg/mL, which was significantly lower ($P \leq 0.05$) than the control sample (T0) (18.20±1.07 mg/mL). This indicates that the formulated biscuits had significantly higher antioxidant activity because of the presence of walnut powder.

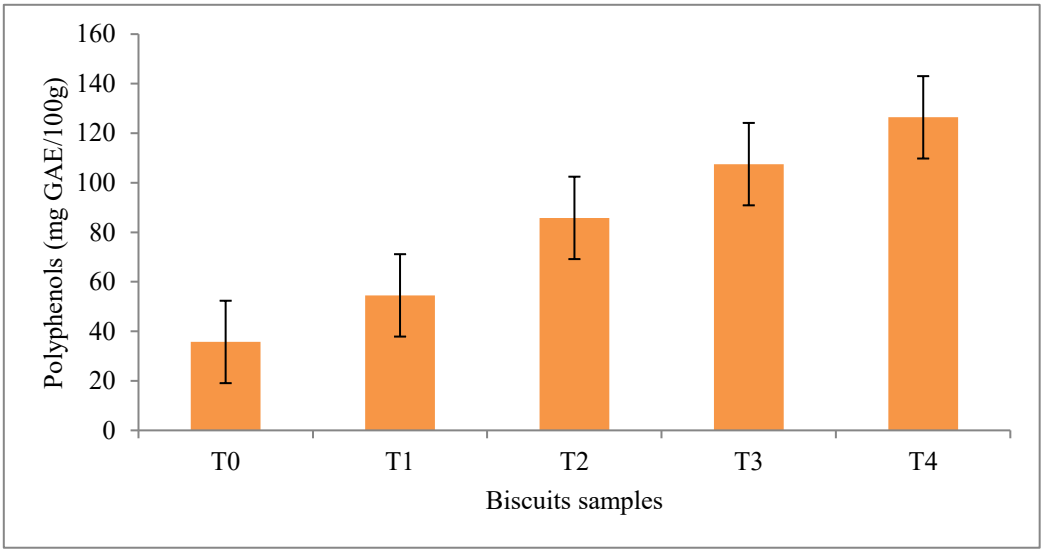


Fig. 1 Total polyphenols contents of control and walnut rich (omega-3) formulated biscuits

Discussion

Walnuts are rich in polyunsaturated fatty acids (PUFA), especially linolenic and linoleic acids. Walnuts' high PUFA content (72.6–78.0% of total fatty acids) reduces the risk of heart disease by increasing HDL cholesterol and decreasing LDL cholesterol. Minerals (potassium (300-370 mg 100 g^{-1}), calcium (83-135 mg 100 g^{-1}), magnesium (381-443 mg 100 g-1), melatonin (1.0-1.9 ng g^{-1}), serotonin (10-34 ng g^{-1}), proteins (15.1-17.4 g 100 g^{-1}), and dietary fiber (4.2-5.2 g 100 g^{-1}) (Cerit et al., 2017). Large amounts of digestible, dry-based proteins are found in walnuts and are beneficial to human nutrition, particularly for vegetarians. Due to presence of all essential nutrients in the walnuts it is a good functional food (Romano et al., 2021). The higher fat content that results from replacing wheat flour with walnut powder may be the cause of the low sensory scores of textures between prepared biscuits. This can also lead to low aroma and overall acceptability, as demonstrated by new biscuits made with additions from various sources (Khalil et al., 2023). In particular, samples added with 2% and 5% walnut powder did not exhibit significant variations in any of the examined sensory qualities, including appearance, surface and interior color, texture, scent, taste, and overall acceptance of biscuits. The only aftertaste aspects that differed substantially for the biscuits enhanced with 5% walnut powder were interior color, texture, taste, and overall acceptability; the panelists gave these samples higher marks than control biscuits ($P<0.05$). In summary, all prepared biscuit samples received high scores from the panelists, with biscuits created with 5% walnut powder having a higher overall acceptance ($P<0.05$).

The use of nutraceuticals (plant extract/food commodities) in biscuit formulation to increase polyphenols has been the subject of numerous similar studies (Dhankhar et al., 2019; Conforti et al., 2021; Lou et al., 2022). According to several researches, foods enriched in polyphenols have an impact on blood lipid levels by raising HDL and decreasing LDL (Potì et al., 2019). Due to the addition of walnut powder, which has a higher concentration of phenolic compounds than other nuts (Pop et al., 2021), due to walnuts T3 and T4 have high polyphenol content. According to a study by Amen et al. (2023), adding walnuts to a normal diet can enhance the amount of polyphenols consumed since nuts can raise the overall amount of polyphenols in the human diet. As a result, both biscuit manufacturers and customers benefit greatly from the panelists' highest-scoring biscuits that have been sensory enhanced with walnut powder. Sensory study of newly developed biscuits is crucial since any food sensory feature can have a big impact on customers' decisions to suggest a product for the market based on its overall acceptability and nutritional value (Bölek, 2022). It is important to determine the appropriate amounts of walnut powder used, as this could change consumers' perceptions of goods with the best sensory evaluation. Furthermore, considering the growing consumer demand for antioxidant-rich foods that offer potential health benefits without compromising their sensory attributes (Arslan et al., 2019; Dhillon et al., 2025).

The DPPH assay, which is very accurate, rapid, authenticated, and simple to use, was used to assess the antioxidant properties of the biscuit samples by determining their radical scavenging activities (Saeed et al., 2025). Our findings are consistent with research showing that adding highly polyphenolic products increased antioxidant activity (Garcia-Mendoza et al., 2021; Romano et al., 2021; Kumar et al., 2023). According to Sik et al. (2024), biscuits' antioxidant qualities and characterization may result in higher levels of total polyphenol and total flavonoids as well as DPPH radical scavenging capabilities. Because these phenolic compounds have been shown a number of biological activities, including antioxidative activity, heart disease prevention, and anti-inflammatory qualities (Nguyen et al., 2023; Saeed et al., 2026a,b), they may serve as a defense mechanism against infections and parasites.

Hydroxyl-substituted aromatic frameworks serve as the structural foundation for phenolic antioxidants, which stabilize phenoxy radicals through resonance delocalization. These bioactive substances exhibit protective benefits through a variety of methods, including prophylactic suppression of radical initiation or redox-based inactivation of free radicals and their precursors through electron or hydrogen atom donation (Wang et al., 2026). Because the study's use of walnut powder demonstrated exceptionally high antioxidant activities, these effects were included into the biscuits. Therefore, it is anticipated that the antioxidant-rich cookies created in this study will be beneficial to customers' general health. The improved nutritional and antioxidant qualities of biscuits found in this study are healthier and valuable in food market. The developed biscuit are rich in polyphenols, due to use of walnut powder which has an excellent source of the necessary nutrition and useful bioactive substances that improve general human health (Wibowo et al., 2024). According to Akpoko et al. (2026), biscuits are thought to be a great food carrier matrix for incorporating nutraceutical components and show enhanced functionality in terms of physical, micronutrient, and antioxidant qualities. All age groups have come to love baked goods like biscuits, cookies, pasta and noodles (Gwak et al., 2026; Adeyemo et al., 2026). One of the most popular baked goods is biscuits, which can be replaced with nutrients that are bioactive and useful. These can be utilized as a very simple means of supplying the people with an adequate quantity of nutrients. Walnuts are a good source of bioactive nutrients and can be used to develop new food products or strengthen existing ones (Jung et al., 2026). For consumers' overall sensory acceptance, more research on the development of other nuts products is advised.

Conclusion

In conclusion, the current study revealed that by adding walnut powder in biscuits improved their nutritional value, particularly omega-3 fat. The formulated biscuits' sensory evaluation and antioxidant potential were improved by adding 2.5, 5.0, 7.5 and 10% walnut powder. By adding 7.5% walnut powders being the most beneficial with respect to its overall acceptability. Consequently, it is advised to use walnut powder when making biscuits in order to target the nutritional values, sensory parameters and antioxidant properties. These findings suggest that walnut powder has important health benefits for human health conditions and may be used as a source of natural antioxidants and omega-3 fat.

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Data availability: Upon reasonable request, the relevant author will provide the data supporting the study's conclusions.

Declarations

i. Ethics clearance and permission to take part: Every individual participant in the study's sensory examination provided ethical approval. No personally identifiable information was included in the experimentally gathered data used in this study. The authors affirm that this is their own original work that hasn't been published anywhere else.

ii. Conflicts of interest: The authors have no any conflicts of interest.

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References

Abedin, M. J., Akter, S., Chowdhury, A. S., Ahmed, M. M., & Farzana, T. (2026). Physicochemical, sensory, and antioxidant properties of wheat biscuits fortified with soy flour and guava (*Psidium guajava*) powder. *Legume Science, 8*(2), e70127. https://doi.org/10.1002/leg3.70127

Abedin, M. J., Akter, S., Chowdhury, A. S., Ahmed, M. M., & Farzana, T. (2026). Physicochemical, sensory, and antioxidant properties of wheat biscuits fortified with soy flour and guava (*Psidium guajava*) powder. *Legume Science, 8*, e70127. https://doi.org/10.1002/leg3.70127

Adeyemo, I. M., Laleye, P. O., & Falade, K. O. (2026). Enhancing pasta quality through substitution of wheat with acha, sorghum and *Cirina forda* powder. *Journal of*

Food Measurement and Characterization, 30, 1–17. https://doi.org/10.1007/s11694-026-04074-7

Akpoko, F. B., Abong, G. O., & Njue, L. G. (2026). Sensory and nutritional evaluation of biscuits from blends of wheat flour, wheat pollard flour and defatted soy flour. *African Journal of Food, Agriculture, Nutrition and Development, 26*(1), 28509–28531. https://doi.org/10.18697/ajfand.148.26140

Akuirene, J. O., Itam, V. E., Ibe, M. O., Johnny, J. O., Mkpat, J. F. E., Ene-Obong, H. N., & Williams, I. O. (2026b). Sensory evaluation and nutrient composition of biscuits produced from composite flour of yellow maize (*Zea mays*) and Coconut (*Cocos nucifera*). *South Asian Research Journal of Natural Products, 9*(2), 500–514. https://doi.org/10.9734/sarjnp/2026/v9i2258

Akuirene, J. O., Oko, G. E., Egom, E. O., Ogar, F. A., & Edide, U. J. (2026a). Nutrient composition of locally compounded enteral feed powders from soya bean (*Glycine max*), finger millet (*Eleusine coracana*), and red sorghum (*Sorghum bicolor*). *South Asian Research Journal of Natural Products, 9*(1), 18–33. https://doi.org/10.9734/sarjnp/2026/v9i1222

Al-Subaie, F. A., & Shori, A. B. (2026). Fermentation as a strategy to improve the bioactive and antioxidant properties of walnuts and their derived products. *Discover Applied Sciences, 8*, 188. https://doi.org/10.1007/s42452-025-08185-6

Amen, R. I., Sirirat, R., Oda, K., Rajaram, S., Nwachukwu, I., Cofan, M., Ros, E., Sabaté, J., & Haddad, E. H. (2023). Effect of walnut supplementation on dietary polyphenol intake and urinary polyphenol excretion in the walnuts and healthy aging study. *Nutrients, 15*(5), 1253. https://doi.org/10.3390/nu15051253

AOAC International. (2019). *Official methods of analysis of AOAC International* (21st ed.; Method 925.10). AOAC International. Published by the Association of Official Analytical Chemists, USA. https://www.aoac.org/official-methods-of-analysis/

Arslan, M., Rakha, A., Xiaobo, Z., & Mahmood, M. A. (2019). Complimenting gluten free bakery products with dietary fiber: Opportunities and constraints. *Trends in Food Science and Technology*, *83*, 194–202. https://doi.org/10.1016/j.tifs.2018.11.011

Bakar, M. F. A., Ranneh, Y. & Kamil N. F. M. (2022). Development of high fiber rich antioxidant biscuits from purple and orange sweet potato peels. *Food Research, 6*(1), 12–19. https://doi.org/10.26656/fr.2017.6(1).036

Bereda, G., Ahamad, J., & Ahmed, H. A. (2026). Phytopharmacological activities and translational evidence of Juglans regia L. from experimental studies to clinical insights. *Discover Food, 6*, 304. https://doi.org/10.1007/s44187-026-01111-8

Bölek, S. (2022). Valorization of roasted longan stone in production of functional biscuits with high antioxidant activity and dietary fiber. *Food Science and Technology*, *42*, e69820. https://doi.org/10.1590/fst.69820.

Brand-Williams, W., Cuvelier, M. E., & Berset, C. L. (1995). Use of a free radical method to evaluate antioxidant activity. *LWT-Food science and Technology, 28*(1), 25-30. https://doi.org/ 10.1016/S0023-6438(95)80008-5

Castillo-López, R. I., León-Félix, J., Angulo-Escalante, M. Á., Gutiérrez-Dorado, R., Muy-Rangel, M. D., & Basilio Heredia, J. (2017). Nutritional and phenolic characterization of *Moringa oleifera* leaves grown in Sinaloa, México. *Pakistan Journal of Botany, 49*(1), 161-168.

Cerit, İ., Sariçam, A., Demirkol, O., Ünver, H., Sakar, E., & Coşansu, S. (2017). Comparative study of functional properties of eight walnut (*Juglans regia* L.) genotypes. *Food Science and Technology, 37*(3), 472–477. https://doi.org/10.1590/1678-457X.20516

Conforti, P. A., & Patrignani, M. (2021). Increase in the antioxidant content in biscuits by infusions or *Prosopis chilensis* pod flour. *Open Agriculture, 6*, 243–253. https://doi.org/10.1515/opag-2021-0016

Cui, M., Mo, R., Li, Q., Wang, R., Shen, D., Tang, F., & Liu, Y. (2024). Maturation-induced changes in phenolic forms and their antioxidant activities of walnuts: A dual view from kernel and pellicle. *Food Chemistry: X, 23*, 101792. https://doi.org/10.1016/j.fochx.2024.101792

Dhankhar, J., Vashistha, N., & Sharma, A. (2019). Development of biscuits by partial substitution of refined wheat flour with chickpea flour and date powder. *Journal of Microbiology, Biotechnology and Food Sciences, 8*(4), 1093–1097. https://doi.org/10.15414/jmbfs.2019.8.4.1093-1097

Dhillon, P. K., Tanwar, B., Jayprakash, R. P., & Singla, P. (2025). Micronutrient, total phenol content and total antioxidant activity of biscuits with assorted nutraceuticals. *International Journal of Food Science and Nutrition, 10*(1), 21–26.

Fekete, M., Lehoczki, A., Kryczyk-Poprawa, A., Zábó, V., Varga, J. T., Bálint, M., Fazekas-Pongor, V., Csípő, T., Rząsa-Duran, E., & Varga, P. (2025). Functional foods in modern nutrition science: Mechanisms, evidence, and public health implications. *Nutrients, 17*(13), 2153. https://doi.org/10.3390/nu17132153

Feumba, D. R., Atud, S. E., Panyoo, A. E., Metsatedem, T. Q., & Tchuenchieu, A. D. (2026). Formulation of nutrient-enriched biscuits from wheat, pumpkin, and yellow maize composite flours using mixture design. *Journal of Food Processing and Preservation, 2026*, 5495706. https://doi.org/10.1155/jfpp/5495706

Fukasawa, R., Miyazawa, T., Abe, C., Bhaswant, M., & Toda, M. (2024). Functional components of walnuts: A review focusing on native and cultivated species. *Food Science and Technology Research, 30*(1), 1–12. https://doi.org/10.3136/fstr.FSTR-D-23-00093

Garcia-Mendoza, M. del P., Espinosa-Pardo, F. A., Savoire, R., Etchegoyen, C., Harscoat-Schiavo, C., & Subra-Paternault, P. (2021). Recovery and antioxidant activity of phenolic compounds extracted from walnut press-cake

using various methods and conditions. *Industrial Crops and Products, 167*, 113546. https://doi.org/10.1016/j.indcrop.2021.113546

Gwak, N., Zogona, D., Balandrano, H. D. D., & Beta, T. (2026). Physicochemical properties and antioxidant activity of wild rice–wheat noodles enriched with purple sweet potato powder. *Journal of Food Biochemistry, 2026*, 8840900. https://doi.org/10.1155/jfbc/8840800

Hsu, C.-Y., Alzahrani, A. A., Maabreh, H. G., Prasad, K. D. V., Bokov, D. O., Kareem, A. H., Alawadi, A., Ihsan, A., Shakir, M. N., & Alasheqi, M. Q. (2024). Effect of walnut consumption on markers of endothelial function in adults: A systematic review and meta-analysis of randomized controlled trials. *Phytotherapy Research, 38*(3), 1358–1366. https://doi.org/10.1002/ptr.8109

Jiao, R., Zhang, F., Yang, L., & Wang, C. (2026). Development of grape seed extract-fortified biscuits: Effects on dough rheology, biscuit quality, antioxidant activity, and sensory evaluation. *Foods, 15*, 2944. https://doi.org/10.3390/foods15162944

Jung, H. S., Yoo, H. W., & Kim, C. W. (2026). Effect of foliar fertilization on the antioxidant activity of walnut (Juglans regia L.) kernels. *Forest Science and Technology, 22*(2), 376–385. https://doi.org/10.1080/21580103.2026.2621890

Khalil, N., Elbeltagy, A. E., Aljutaily, T., Ali, A., & Gadallah, M. (2023). Organoleptic, antioxidant activity and microbial aspects of functional biscuit formulated with date fruit fibers grown in Qassim Region. *Food Science and Technology, 43*, e95222. https://doi.org/10.1590/fst.95222

Kumar, P., Székely, D., Szalóki-Dorkó, L., Kereszturi, J., & Máté, M. (2023). Phenolic profile, color parameters and antioxidant activity of walnut kernel extracts as influenced by different time and temperature during extraction. *Progress in Agricultural Engineering Sciences, 19*(S1), 87–95. https://doi.org/10.1556/446.2023.00086

Lou, W., Zhou, H., Li, B., & Nataliya, G. (2022). Rheological, pasting and sensory properties of biscuits supplemented with grape pomace powder. *Food Science and Technology (Campinas)*, *42*, e78421. http:// dx.doi.org/10.1590/fst.78421

Ma, Z. F., Liu, S., Fu, C., Zhou, S., & Lee, Y. Y. (2026). Functional foods in health promotion and disease prevention: Innovations, evidence and challenges. *Foods, 15*(4), 764. https://doi.org/10.3390/foods15040764

Manful, C. F., Fordjour, E., Subramaniam, D., Sey, A. A., Abbey, L., & Thomas, R. (2025). Antioxidants and reactive oxygen species: Shaping human health and disease outcomes. *International Journal of Molecular Sciences, 26*(15), 7520. https://doi.org/10.3390/ijms26157520

Mashayekhi, G., Ashtary-Larky, D., Karimi, M., Asbaghi, O., Porkar Rezaeyeh, A., Shouhani, Z., Hosseini, A., & Naderian, M. (2026). Effects of walnut consumption on blood lipid profile and apolipoproteins in adults: A GRADE-assessed systematic review and dose–response meta-analysis of 49 randomized controlled trials. *Food Science & Nutrition, 14*(2), e71526. https://doi.org/10.1002/fsn3.71526

Najjar, Z., Kizhakkayil, J., Shakoor, H., Platat, C., Stathopoulos, C., & Ranasinghe, M. (2022). Antioxidant potential of cookies formulated with date seed powder. *Foods, 11*(3), 448. https://doi.org/10.3390/foods11030448

Nguyen, T. H. D. & Vu, D. C. (2023). A review on phytochemical composition and potential health-promoting properties of walnuts. *Food Reviews International*, *39*(1), 397–423. https://doi.org/10.1080/87559129.2021.1912084.

Ojewumi, E. O., Omoba, S. O., & Awolu, O. O. (2021). Antioxidant and antinutritional properties of biscuit produced from enzymatically modified tacca (*T. involucrata*) flour. *Technology in Nutrition and Food Science, 6*(2), 000632. https://doi.org/10.31031/NTNF.2021.06.000632

Pop, A., Fizeșan, I., Vlase, L., Rusu, M. E., Cherfan, J., Babota, M., Gheldiu, A., Tomuta, I., & Popa, D.-S. (2021). Enhanced recovery of phenolic and tocopherolic compounds from walnut (*Juglans regia* L.) male flowers based on process optimization of ultrasonic-assisted extraction: Phytochemical profile and biological activities. *Antioxidants, 10*(4), 607. https://doi.org/10.3390/antiox10040607

Potì, F., Santi, D., Spaggiari, G., Zimetti, F., & Zanotti, I. (2019). Polyphenol health effects on cardiovascular and neurodegenerative disorders: A review and meta-analysis. *International Journal of Molecular Sciences, 20*(2), 351. https://doi.org/10.3390/ijms20020351

Qin, G., Li, Y., Sun, J., Wang, Z., Nie, J., Kong, K. W., Ismail, A., Si, Y., & Zhang, X. (2026). Utilization of walnut male flowers (*Juglans regia* L.) as a functional ingredient in biscuits: Impacts on dough rheology, nutritional composition, glycemic response, and sensory attributes. *Food Science & Nutrition, 14*(2), e71510. https://doi.org/10.1002/fsn3.71510

Qingyang, L., Shuting, W., Ruohui, W., Danyu, S., Runhong, M., Fubin, T., & Yihua, L. (2024). Comparative investigation on the phenolic compounds and antioxidant capacity of walnut kernel from different drying methods. *Food Production, Processing and Nutrition, 6*, 41. https://doi.org/10.1186/s43014-023-00216-2

Rabrenović, B., Natić, M., Dabić Zagorac, D., Meland, M., & Fotirić Akšić, M. (2022). Bioactive phytochemicals from walnut (Juglans spp.) oil processing by-products. In M. F. Ramadan Hassanien (Ed.), *Bioactive phytochemicals from vegetable oil and oilseed processing by-products*. Springer. https://doi.org/10.1007/978-3-030-63961-7_25-1

Rahbardar, M. G., Rashki, M., & Boskabady, M. H. (2025). A review of antioxidant activity, anti-inflammatory properties, apoptosis-regulatory effects, and immune

system modulation of *Juglans regia* L. (walnut). *Food Science & Nutrition, 13*(11), e71081. https://doi.org/10.1002/fsn3.71081

Rashki, M., Ghasemzadeh Rahbardar, M., & Boskabady, M. H. (2025). Nutritional advantages of walnut (Juglans regia L.) for cardiovascular diseases: A comprehensive review. *Food Science & Nutrition, 13*(1), e4526. https://doi.org/10.1002/fsn3.4526

Romano, R., Aiello, A., Meca, G., Deluca, L., Pizzolongo, F., & Masi, P. (2021). Recovery of bioactive compounds from walnut (*Juglans regia* L.) green husk by supercritical carbon dioxide extraction. *International Journal of Food Science & Technology, 56*(9), 4658–4668. https://doi.org/10.1111/ijfs.15161

Saeed, M. K., Zahra, N., Anwar, H. K., Khan, A., Ahmad, I., & Shahzad, K. (2026b). Valorization of mango (*Mangifera indica*) peel waste as a functional nutritional resource with antioxidant potential: Valorization of mango (*Mangifera indica*) peel waste with antioxidant potential. *Diet Factor, 7*, 22-26. https://doi.org/10.54393/df.v7i01.203

Saeed, M. K., Zahra, N., Rasheed, A. A., Ijaz, A., Shehzad, K., Ahmad, I., & Abdi, S. H. I. (2026a). Determination of nutritional facts, polyphenolic content and radical scavenging activity of black pepper (*Piper nigrum* L.) from Pakistan. *Diet Factor, 7*(2), 49–54. https://doi.org/10.54393/df.v7i2.221

Saeed, M. K., Zahra, N., Saeed, A., Syed, Q., & Abidi, S. H. (2024). Banana peels: A contemptible source of dietary fiber and natural antioxidants. *Acta Pharmaceutica Sciencia, 62*(1), 89–103. https://doi.org/10.23893/1307-2080.APS6206

Saeed, M. K., Zahra, N., Saeed, A., Syed, Q., & Abidi, S. H. (2025). Physico-phytochemical exploration, antiradical and reducing power potential of oleanderum leaves. *Bangladesh Journal of Scientific and Industrial Research*, *60*(2), 107–114. https://doi.org/10.3329/bjsir.v60i2.79782

Sik, B., Ajtony, Z., Lakatos, E., Gál, L. H., & Székelyhidi, R. (2024). Evaluation of the physical, antioxidant, and organoleptic properties of biscuits fortified with edible flower powders. *Food Science & Nutrition*, *12*, 3265–3272. https://doi.org/10.1002/fsn3.3993

Singleton, V. L., Orthofer, R., & Lamuela-Ravento's, R. M. (1999). Analysis of total phenols and other oxidation substrates and antioxidants by means of Folin-Ciocalteu reagent. *Methods in Enzymology*, *299*, 152-178. http://doi.org/10.1016/S0076-6879(99)99017-1

Wang, X., Gao, Z., Yang, L., Ren, J., & Wang, C. (2026). Grape seed extract fortification: Effects on dough properties and quality attributes of fresh wet noodles from medium-gluten wheat flour. *Foods, 15*, 1400. https://doi.org/10.3390/foods15081400

Wibowo, S. A., & Surono, I. S. (2024). Phenolic content and antioxidant activity of formulated biscuits with banana, tempeh and moringa flours. *Brazilian Journal of Food Technology, 27*, e2023068. https://doi.org/10.1590/1981-6723.06823

Yadav, K. C., Bhattarai, S., Shiwakoti, L. D., Paudel, S., Subedi, M., Pant, B. R., Paudel, M., Dhugana, S., Bhattarai, S., Tiwari, T., Koirala, N., & Upadhyaya, J. (2022). Sensorial and chemical analysis of biscuits prepared by incorporating *Moringa* flower powder and leaf powder. *International Journal of Food Properties*, *25*, 894–906. https://doi.org/ 10.1080/ 10942 912.2022.2069807

Zhang, Y., Cai, P., Cheng, G., & Zhang, Y. (2022). A brief review of phenolic compounds identified from plants: Their extraction, analysis, and biological activity. *Natural Product Communications, 17*, 1–14. https://doi.org/10.1177/1934578X21106972

Zhao, C.-R., Chen, Q., Ma, X., Yang, T.-Y., Yan, W.-J., Rong, R.-F., & Wang, F. (2024). The chemical composition of the walnut pellicle and its benefits to health. *Food & Medicine Homology, 1*(1), 9420007. https://doi.org/10.26599/FMH.2024.9420007

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