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# Micronutrient, total Phenol content and total antioxidant activity of Biscuits with assorted Nutraceuticals

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**Abstract**
**Background:** In contemporary times, biscuits become inevitable parts of our dietaries. These contain refined wheat flour which is generally lacking in vitamins and minerals.
**Methods:** Three wheat flour-based treatments i.e. T1, T2 and T3 containing 60, 50 and 40% of Defatted soya flour (DSF) to wheat flour (WF) were standardized and evaluated against T0 (100% WF). To develop biscuits with assorted nutraceuticals (T4 and T5), 50% of DSF was replaced with blend of nutraceutical ingredients including 7% of wheat bran. This study aimed to compare the vitamin, mineral, total phenol content and total antioxidant activity of developed biscuits.
**Results:** T5 with tomato-tangerine peel powder had the highest content of vitamin A, C and E, respectively. Calcium and phosphorus content was the highest in T1 while iron and zinc contents were higher in T5 and T4, correspondingly. A significant difference ($p<0.05$) in total phenol content and total antioxidant activity was observed between nutraceutical ingredients with the highest total antioxidant activity in tangerine peel powder. Hence, the same was recorded higher in T5 than other treatments. Nutraceutical blend with tangerine peel powder and tomato pulp along with 7% wheat bran should be included as functional ingredients for biscuits containing 60% WF.

**Keywords:** Biscuits, assorted nutraceuticals, defatted soya flour, mineral content, vitamin content, total phenol content, total antioxidant activity

## Introduction

The most commonly consumed food products are biscuits and breads the world over, especially in developing countries. Biscuits, being leading snack market with present turnover of $76.385 billion, are projected to attain USD 121 and 164 billion in the year 2021 and 2024, respectively, having annual growth rate of 3.7 and 5.08%, correspondingly (Apeda agri exchange, 2020) [7]. Biscuits occupy a noticeable position among all bakery products that accounts 30%, with 28% as highest record of consumption in Northern India followed by Western (25%), Eastern (24%) and Southern (23%) regions of the country, respectively (Ahmad and Ahmed, 2014) [4]. These have become inevitable parts for interims between main meals as they provide the key nutrients i.e. proteins and carbohydrates, however with small amounts of micronutrients such as vitamins and minerals. Relying on biscuits, particularly, is attributed to its extended shelf life, presenting it as the fittest alternative for nutritional upgrading via fortification or supplementation.

Several new trends are emerging to the food industry in contemporary era, with major emphasis on nutraceutical enriched food production, since a constant increase in awareness level of the consumers was found for nutrition security. The incorporation of combination of whole grains and many fruits and vegetable components can assure the improved nutritional profile of final product as these are concentrated and significant sources of dietary fiber and phytoestrogens. Among fruit parts, half of the citrus fruit weight (citrus peel) is considered inedible (Sharma *et al.*, 2017) [24]. Contrarily, these peels have great medicinal value as these are potent antioxidants as being rich in phenolic compounds (Ademosun, 2022) [2] and better free radical scavenging capacity as compared to fruit pulp, thus are used as additives in food and nutraceutical processing setups (Rafiq *et al.*, 2018) [21].

Apart from this, combination of these food ingredients has been considered as an effective remedy for their increased total antioxidant activity. Though, there is robust evidences of improvement regarding nutrient profile, on inclusion of DSF to whole wheat flour (WWF) while developing cookies/ biscuits (Olapade and Adeyemo, 2014; Zaker *et al.*, 2012; Youssef and Mousa, 2012) [20, 29, 30] but products available in the market are consisting fewer amounts of bioactive components (only one or two), with no multiple nutraceutical blending samples. Additionally, considering increased market demand for antioxidant rich foods, citrus waste can be worked out to develop novel food products, since these peels are considered as safe products with proven health benefits without altering sensory attributes (Guine *et al.*, 2020). The present study aimed at investigating the efficacy of nutraceutical supplementation on augmented micronutrient composition, phenol content and total antioxidant activity of developed biscuits.

## Materials and methods

**Development of Biscuits:** Development of the biscuits with assorted nutraceuticals has been represented in Figure 1. This research work was conducted during the year 2018-19 at Food Technology Laboratory, Lovely Professional University, phagwara.

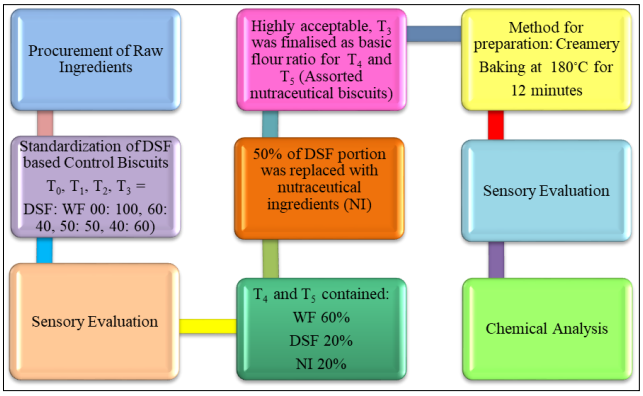


**Fig 1:** Development and Evaluation of biscuits

**Chemical analysis:** Vitamins (vitamin A (AOAC, 1980), vitamin C (AOVC, 1996) [6] & vitamin E (Emmerie and Engel, 1938) [11] were estimated in raw material, DSF based biscuits and biscuits with assorted nutraceuticals. Further, minerals (calcium, phosphorus, iron and zinc) were analysed using Inductively Coupled Plasma Atomic Emission Spectroscopy method (Dilek *et al.*, 2011) [10]. Total phenol content and total antioxidant activity of above-mentioned samples were also determined according to the standard method (Singleton *et al.*, 1999; Brand-Williams *et al.*, 1995) [9, 25].

**Statistical analysis:** The recorded data was analysed using Tukey's test, One-way ANOVA (analysis of variance) to study the significant difference ($p<0.05$) between all the parameters recorded using the software GraphPad Prism version 5.01.

## Results

**Vitamin content:** Vitamin content of nutraceutical ingredients and developed biscuit treatments has been presented in Table 1.

**Table 1:** Vitamin content of nutraceutical ingredients and biscuits

| Samples | Vitamin-A (µg/100g) | Vitamin-C (mg/ 100 g) | Vitamin-E (mg/ 100 g) |
|---|---|---|---|
| Ingredient Sample | | | |
| Defatted Soya Flour | 402±1.98b | 0.0±0.0 | 0.07±0.01d |
| | | | |
| Wheat Flour | 19.7±0.2e | 0.0±0.0 | 0.0±0.0 |
| Wheat Bran | 0.0±0.0 | 0.0±0.0 | 0.71±0.03b |
| Tomato | 144±1.23c | 26.1±0.05b | 0.91±0.06a |
| Tangerine Peel | 554.2±4.14a | 28.7±1.24a | 0.26±0.02c |
| | | | |
| Ginger | 24.2±0.37d | 3.1±0.12c | 0.0±0.0 |
| Biscuit Samples | | | |
| $T_0$ | 115.2±6.2f | 0.0±0.0 | 0.0±0.0 |
| | | | |
| $T_1$ | 1110.4±6.2c | 0.0±0.0 | 0.04±0.0c |
| $T_2$ | 1067.1±4.2d | 0.0±0.0 | 0.03±0.0d |
| $T_3$ | 1028.9±8.6e | 0.0±0.0 | 0.02±0.0e |
| | | | |
| $T_4$ | 1119.2±4.9b | 4.7±0.42b | 1.30±0.2b |
| | | | |
| $T_5$ | 1331.5±7.6a | 5.6±0.30a | 1.32±0.31a |

*Values are Mean±SD from triplicate determinations; different superscripts in the same column are significantly different ($p<0.05$)

Where $T_0$= 100% Wheat Flour
$T_1$= 60% Defatted Soya Flour and 40% Wheat Flour
$T_2$= 50% Defatted Soya Flour and 50% Wheat Flour
T3 = 40% Defatted Soya Flour and 60% Wheat Flour
$T_4$= 20% Defatted Soya Flour, 60% Wheat Flour, 2% Ginger (Powder),7% Wheat Bran,10% Tomato pulp, 0.5% Turmeric and 0.5% Black pepper
$T_5$=20% Defatted Soya Flour, 60% Wheat Flour, 2% Tangerine peel (Powder), 7% Wheat Bran, 10% Tomato pulp, 0.5% Turmeric and 0.5% Black pepper

**Vitamin content of nutraceutical ingredients:** Vitamin A content ranged from 19.7 to 554.2µg/100g in nutraceutical ingredients. Defatted soya flour and tangerine peel powder (402 and 554.2µg/100g, respectively) had higher vitamin A than in ginger and wheat flour (24.2 and 19.7µg) whereas vitamin A content was absent in wheat bran. Vitamin C content was 3.1, 26.1 and 28.7mg in ginger, tomato and tangerine peel powder, respectively while this vitamin was not present in wheat flour, defatted soya flour and wheat bran. Vitamin E content was ranged from 0.0 to 0.91mg in nutraceutical ingredients. It was present as 0.91 and 0.71mg per100g in tomato and wheat bran, respectively. In contrast to this, both refined wheat flour and ginger had no vitamin E content. Figures obtained for vitamin content of nutraceutical ingredients were close to the reference values by Gopalan *et al.*, 2004 [14].

**Vitamin content of biscuits:** Vitamin A content of control and biscuits with assorted nutraceutical samples was significantly ($p<0.05$) different. Among control biscuit samples, T1 had the highest (1110.4µg) vitamin A content as compared to T2 (1067.1µg) and T3 (1028.9µg), respectively. It was reported as 1119.2 and 1331.5µg in T4 and T5, respectively whereas T0 had lowest value of vitamin A as 115.2 µg. Further, vitamin C was not present in all the control samples whereas biscuits with assorted nutraceuticals i.e. T4 and T5 had 4.7 and 5.6mg of vitamin C, respectively. The mean vitamin E was 0.04, 0.03 and 0.02mg in T1, T2 and T3, respectively, whereas it was 1.30 and 1.32mg in T4 and T5, respectively. No amount of vitamin C and E was detected in T0. A significant ($p<0.05$) difference was observed among all the biscuit treatments for vitamin A content. T1 had the highest vitamin A content as

compared to T2 and T3, respectively. Higher vitamin A content in T5 attributed to combination of defatted soya flour and tangerine peel powder in it. Vitamin C was not present in the control treatments as cereal and legume flours contain no vitamin C (Gopalan *et al.*, 2004) [14]. On the contrary, biscuits with assorted nutraceuticals, T4 and T5 had good amounts of vitamin C, respectively, due to presence of tomato and tangerine peel powder. Both vitamin C and E were not detected in T0, whereas good amounts of vitamin C and E were recorded in T4 and T5, samples, due to incorporation of vitamin C (tomato and tangerine peel powder) and vitamin E (whole wheat flour and wheat bran) rich ingredients in biscuits with assorted nutraceuticals.

**Mineral content:** Calcium, phosphorus, iron and zinc contents were estimated in nutraceutical ingredients and biscuit treatments are shown in Table 2.

**Table 2:** Mineral content of nutraceutical ingredients and biscuits

| Samples | Calcium (mg/ 100 g) | Phosphorus (mg/ 100 g) | Iron (mg/ 100 g) | Zinc (mg/ 100 g) |
|---|---|---|---|---|
| Ingredient Samples | | | | |
| Defatted Soya Flour | 201.4±1.2a | 663.4±1.56b | 6.7±0.8b | 2.1±0.12b |
| Wheat Flour | 18.9±0.61e | 108.9±0.33c | 1.9±0.2e | 0.45±0.07e |
| Wheat Bran | 34.6±0.45d | 799.2±0.66a | 4.15±0.21c | 1.85±0.1c |
| Tomato | 12.14±0.56f | 31.6±0.17e | 1.1±0.04f | 0.0±0.0f |
| Tangerine Peel | 157.3±1.16b | 17.1±0.31f | 7.87±0.4a | 6.96±0.34a |
| Ginger | 61.2±0.32c | 53.8±0.42d | 2.7±0.3d | 0.67±0.03d |
| Biscuit Samples | | | | |
| $T_0$ | 56.2±2.85f | 209.35±1.95f | 1.46±0.0f | 0.4±0.0f |
| $T_1$ | 474.7±4.30a | 390±4.2c | 4.08±0.07c | 1.68±0.43c |
| $T_2$ | 208.7±1.25d | 377.6±4.6d | 3.1±0.02e | 1.52±0.15d |
| $T_3$ | 174.8±2.10e | 364.1±3.1e | 3.92±0.06d | 1.24±0.30e |
| $T_4$ | 258.4±5.00c | 726.4±7.2a | 6.0±0.23b | 2.76±0.53a |
| $T_5$ | 281.6±3.15b | 717.2±5.0b | 6.7±0.15a | 2.69±0.11b |

*Values are Mean±SD from triplicate determinations; different superscripts in the same column are significantly different ($p<0.05$)

Where $T_0$= 100% Wheat Flour
$T_1$= 60% Defatted Soya Flour and 40% Wheat Flour
$T_2$=50% Defatted Soya Flour and 50% Wheat Flour
T3 =40% Defatted Soya Flour and 60% Wheat Flour
$T_4$= 20% Defatted Soya Flour, 60% Wheat Flour, 2% Ginger (Powder),7% Wheat Bran,10% Tomato pulp, 0.5% Turmeric and 0.5% Black pepper
$T_5$=20% Defatted Soya Flour, 60% Wheat Flour, 2% Tangerine peel (Powder), 7% Wheat Bran, 10% Tomato pulp, 0.5% Turmeric and 0.5% Black pepper

**Mineral content of nutraceutical ingredients:** Regarding mineral content, the mean calcium and phosphorus content was ranged from 12.14 to 201.4 and 17.1 to 799.2mg, respectively. The highest (201.4 and 157.3mg) calcium contents were found in defatted soya flour and tangerine peel powder whereas, lowest (18.9 and 12.14mg) were observed in wheat flour and tomato, respectively. Wheat bran and defatted soya flour had 799.2 and 663.4mg mean phosphorus content, respectively. Further, iron content was ranged from 1.1 to 7.87mg. The iron content was present in fairly good amounts in tangerine peel powder, defatted soya flour and wheat bran, as the corresponding values were 7.87, 6.7 and 4.15mg/100g of above-mentioned ingredients, respectively. The zinc content ranged between 0.0 and 6.96mg in nutraceutical ingredients. Besides, figures for zinc content were recorded as 6.96, 2.1 and 1.85mg in these ingredients, respectively. Mineral content of nutraceutical ingredients were in accordance to the reference nutritive values (Gopalan *et al.*, 2004) [14].

**Mineral content of biscuits:** Calcium content was highest (474.7mg) in T1 followed by T2 (208.7mg) and T3 (174.8mg), respectively. Further, it was present as 258.4 and 281.6mg in T4 and T5, respectively. Higher (390mg) amount of phosphorus was also observed in T1 as compared to rest of the two (377.6 and 364.1mg in T2 and T3) control samples. Phosphorus was detected as 726.4 and 717.2mg in T4 and T5, respectively. The iron content was 4.08, 3.1 and 3.92mg in per 100g of control samples whereas it was found quite higher (6.0 and 6.7mg) in assorted nutraceutical biscuit samples i.e. T4 and T5, respectively. The mean zinc content was 1.68, 1.52 and 1.24mg in T1, T2 and T3 samples, conversely, higher contents of zinc were found in T4 and T5 (2.76 and 2.69mg) in biscuits with assorted nutraceuticals. A significant ($p<0.05$) difference in calcium content was observed between all the biscuit treatments. Phosphorus was observed as high as 726.4 and 717.2mg in T4 and T5, respectively. A significant ($p<0.05$) difference was found in the iron content of control and treatments. The mean iron content was higher in T4 and T5, respectively. The mean zinc content was higher again in these samples. Nevertheless, iron and zinc content was found as low as 1.46 and 0.4mg in T0. Copious developmental studies aiming at improved biscuit nutrition have been conducted previously by intervening the basic wheat flour content with food waste such as fruit peels, wheat bran etc. and reported to have increased nutritional quality in terms of minerals and antioxidants (Adeola and Ohizua, 2018) [2]. Wheat bran is considered as the richest source of calcium, iron and zinc and supplementing wheat bran with soya flour contributed to increased mineral content in biscuits with assorted nutraceuticals during present study. Since, 44.45% of the total vitamins and minerals are present in germ and bran parts of grains (Majzoobi *et al.*, 2014) [19], in the same way, a significantly ($p<0.05$) higher content of vitamins and minerals was recorded in wheat flour and wheat bran as compared to refined wheat flour, in present study. Similar findings were reported by Kumar *et al.*, 2018 [18] as for iron content in biscuits with assorted nutraceutical i.e. T4 and T5, during present investigation. During present study, higher zinc content was in T4 and T5 when compared to

control samples. Reason for that may be wheat bran separated during refining of wheat flour which is the richest source of minerals. Further, Atobatele and Afolabi, 2016 [8] reported the zinc content between 3.25 to 5.35mg/kg of cookies developed using maize and soya flour. Ghoshal and Kaushik, 2020 [12] reported the figures calcium and iron content of cookies supplemented with 25% soymeal as 106 and 6.4mg/100g, respectively whereas the corresponding values were 76.2 and 2.9mg/100g in control cookies samples. Therefore, supplementing whole wheat flour with soya flour contributed to increased mineral content in biscuits with assorted nutraceuticals.

**Total phenol content:** Findings regarding total phenols have been shown in Table 3.

**Table 3:** Total Phenols of nutraceutical ingredients and biscuits

| Samples | Total Phenols (mg GAE/ 100 g) |
|---|---|
| Ingredient Samples | |
| Defatted Soya Flour | 10.8±0.51f |
| Wheat Flour | 164.3±2.49c |
| Wheat Bran | 1203.2±6.48 a |
| Tomato | 221.6±2.54b |
| Tangerine Peel | 31.72±1.02d |
| Ginger | 12.4±0.74e |
| Biscuit Samples | |
| $T_0$ | 105.7±3.79f |
| $T_1$ | 217.4±4.23c |
| $T_2$ | 152.8±4.05d |
| $T_3$ | 124.5±3.15e |
| $T_4$ | 1541±9.60b |
| $T_5$ | 1544±6.92a |

*Values are Mean±SD from triplicate determinations; different superscripts in the same column are significantly different ($p<0.05$)

Where $T_0$= 100% Wheat Flour
$T_1$= 60% Defatted Soya Flour and 40% Wheat Flour
$T_2$=50% Defatted Soya Flour and 50% Wheat Flour
T3 =40% Defatted Soya Flour and 60% Wheat Flour
$T_4$= 20% Defatted Soya Flour, 60% Wheat Flour, 2% Ginger (Powder),7% Wheat Bran,10% Tomato pulp, 0.5% Turmeric and 0.5% Black pepper
$T_5$=20% Defatted Soya Flour, 60% Wheat Flour, 2% Tangerine peel (Powder), 7% Wheat Bran, 10% Tomato pulp, 0.5% Turmeric and 0.5% Black pepper

**Total phenol content of nutraceutical ingredients:** Total phenols (mgGAE/100g) in raw ingredients were ranged from 10.8 to 1203.2mgGAE/100 g. The maximum (1203.2mgGAE/100g) total phenol content was reported in wheat bran followed by tomato (221.6mgGAE/100g) and wheat flour (164.3mgGAE/100g), respectively.

**Total phenol content of biscuits:** With regard to total phenol content, a significant ($p<0.05$) difference was observed between all the treatments. Total phenols were present in amounts of 105.7, 217.4, 152.8, 124.5 and mgGAE/100g in T0, T1, T2 and T3 samples while these amounts were higher in biscuits with assorted nutraceuticals (1541 and 1544 mg GAE/100g in T4 and T5), respectively. Exceptionally higher content of total phenols in biscuits with assorted nutraceutical (T4 and T5) was attributed to addition of herbal nutraceuticals *viz.* ginger, turmeric and tangerine peel powder in their dough mixture. Polyphenol content of biscuits containing 5 and 10% of orange peel powder was 100.73 and 136.66mgGAE/100g, respectively (Rani *et al.*, 2020) [22]. Moreover, these nutraceuticals are having better stability in products prepared at high baking temperature due to formation of maillard reaction products. Total phenolic content increases in baked products due to maillard reaction product formation when baked with addition of baking powder. Adegoke *et al.*, 2017 [1] reported that the total phenolic content was significantly ($p<0.05$) increased in functional biscuits developed by combining wheat flour (70%), soya flour (29.5%) and turmeric powder (0.5%) as compared to control biscuits since the phenolic content was observed as 0.77 and 0.13mgGAE/g in functional and control samples, respectively. Similar findings were reported in another study (Yakoob *et al.*, 2021).

**Total antioxidant activity:** Total antioxidant activity of nutraceutical ingredients and biscuits with assorted nutraceuticals is presented in Figure 2 and 3.

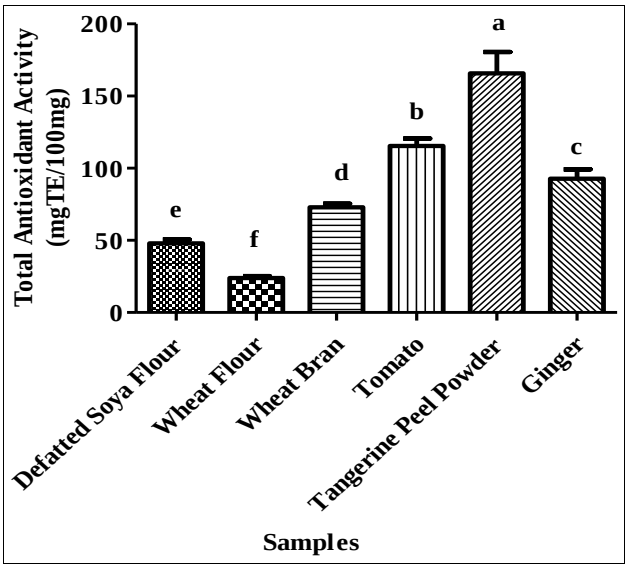


**Fig 2:** Total Antioxidant Activity of nutraceutical ingredients

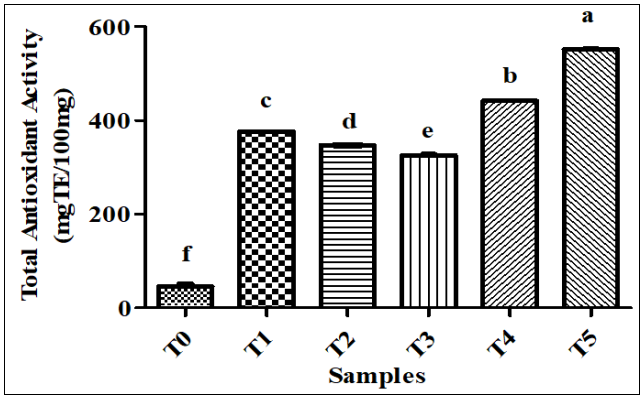


**Fig 3:** Total Antioxidant Activity of biscuit samples

Where T0=100 % Wheat Flour
T1=60 % Defatted Soya Flour and 40 % Wheat Flour
T2=50 % Defatted Soya Flour and 50 % Wheat Flour
T3=40 % Defatted Soya Flour and 60 % Wheat Flour

T4=20 % Defatted Soya Flour, 60% Wheat Flour, 2% Ginger (Powder), 7 % Wheat Bran, 10% Tomato pulp, 0.5 % Turmeric and 0.5 % Black pepper
T5=20 % Defatted Soya Flour, 60 % Wheat Flour, 2% Tangerine peel (Powder), 7 % Wheat Bran, 10% Tomato pulp, 0.5 % Turmeric and 0.5 % Black pepper

**Total antioxidant activity of nutracetical ingredients:** In present study, tomato, tangerine peel powder and ginger have shown more effective total antioxidant activity (>50mgTE/100mg) to quench free radicals as compared to other ingredients. Findings regarding total antioxidant activity revealed a significant ($p<0.05$) difference between nutraceutical ingredients. Tomato, tangerine peel powder and ginger had higher total antioxidant activity to quench free radicals as compared to other ingredients. Whole grains and other plant materials (fruits, vegetables, spices and herbs) possess sufficient amounts of phenolic acids which are known for their excellent antioxidant potential for scavenging free radicals through inhibition of lipid peroxidation.

**Total antioxidant activity of biscuits:** In total antioxidant activity of control and biscuits with assorted nutraceutical samples, a significant ($p<0.05$) difference was observed. The total antioxidant activity was augmented at 10% increased incorporation of defatted soya flour, in case of control treatments. Both the treatments (T4 and T5) of biscuits with assorted nutraceuticals showed higher total antioxidant activity when compared to control samples. Legume flours are full of antioxidants and considered responsible for increasing free radical scavenging activity of the developed product when combined with wheat flour (Thongram *et al.,* 2016) [27]. Besides, addition of nutraceuticals such as tomato, tangerine peel powder, ginger and turmeric in these treatments and production of maillard reaction products during baking enhanced their total antioxidant activity (Rao *et al.,* 2011) [23]. Likewise, increased total antioxidant activity might be attributed to inclusion of nutraceutical blend of legume flour with fruit waste in assorted nutraceutical biscuit treatments during current study. Rani *et al.,* 2020 [22] found significantly ($p<0.05$) increased antioxidant properties in biscuits enriched with citrus peel. The investigators revealed the figures for radical scavenging activity as 26.09 and 33.04mgTE/100g, in biscuits on addition of orange peel at 5 and 10% incorporation level. Supplementation of orange peel powder at 10% incorporation level to wheat flour resulted into increased antioxidant properties and flavonoid content (Thliza *et al.,* 2021) [26]. Total phenol content and DPPH of orange peels were reported as 3.9mgGAE/g and 1.4mgTE, respectively (Gomez-Mejia *et al.,* 2019). Simultaneously, Yaqoob *et al.,* 2021 [28] added dried *kinnow* peel and pomace powder at diverse concentrations (5, 10, 15 and 20%) into wheat flour in preparation of cookies. The investigators studied the functional properties of the prepared cookies and revealed significantly higher values for phenolic content and DPPH in *kinnow* peel supplemented cookies than control.
Conclusively, biscuits are considered an excellent food vehicle matrix for adding nutraceutical ingredients and exhibit improved functionality inform of physical, micronutrient and antioxidant properties (Goubgou *et al.,* 2021) [15].

## Conclusions
It is summarized that improvements in micronutrient content of the cereal-legume based value added products can be made by supplementing the composite flour with other plant materials containing vitamins and minerals. Similarly, ready to eat, value added food products with combination of nutraceutical ingredients will be helpful to enhance the antioxidant status of the product.

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