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Original Article

Evaluation of the Phytochemical and Medicinal Value of Lemongrass (*Cymbopogon citratus*), by Conversion into Powders and Extracts to Develop a Nutritional Bakery Product

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Abstract

Background and objectives: Because of its extraordinary phytomedicinal potential and numerous potential health benefits, lemongrass (*Cymbopogon citratus*), a well-known medicinal and aromatic plant, is of paramount significance. It is typically used as a drug replacement.

Methods: The present study was comprised of drying lemongrass into powder and determining the proximate and mineral composition, and then developing ethanolic extracts of powder to determine total phenolic contents (TPC), total flavonoid contents (TFC), total carotenoids (TC), and DPPH free radical scavenging activity. Next, lemongrass powder (LGP) was replaced at 0, 2.5, 5, 7.5, and 10% levels to develop nutritional biscuits.

Results: The results revealed that lemongrass powder contained higher fiber (8.34 ± 0.04%) and ash (7.26 ± 0.06%) quantities, than wheat flour. Similarly, essential minerals Ca, Mg, K, Fe, and Zn contents in LGP were 36.80 ± 0.12, 64.89 ± 0.13, 54.65 ± 0.18, 12.68 ± 0.05, and 8.46 ± 0.07 mg/100 g dry weight, respectively, which were significantly higher than that calculated in wheat flour. Phytochemical analyses of lemongrass ethanolic extracts documented TPC as 240.46 ± 0.20 mg gallic acid equivalent/100 g, TFC as 98.45 ± 0.15 mg catechin equivalent/100 g, TC as 62.36 ± 0.12 mg/100 g, and DPPH activity as 60.18 ± 0.14 mg AAE/100 g, with such values being significantly higher than those in wheat flour.

Conclusion: Incorporation of LGP at different levels in wheat flour resulted in boosted phytochemical profiles of nutritional biscuits, but upon sensory evaluation of biscuits 2.5% level of LGP provided good scores for taste, flavor and overall acceptability, while for color and flavor 5% LGP was also found to be suitable with highest sensory scores.

Keywords: Lemongrass; Biscuits; Extracts; Nutrition; Phytochemicals; Antioxidants.

Abbreviations: AACC, American Association of Cereal Chemists; AAE, ascorbic acid equivalent; CE, catechin equivalent; ECHA, The European Chemical Agency; DPPH, 2,2-diphenyl-1-picrylhydrazyl; GAE, gallic acid equivalent; GRAS, Generally Recognized as Safe; LGP, lemongrass powder; REACH, Registration, Evaluation, Authorization and Restriction of Chemicals; TPC, total phenolic contents; TFC, total flavonoid contents; TC, total carotenoids.

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Introduction

Lemongrass (*Cymbopogon citratus*) is a warm-season grass, that is a sub-tropical, as well as, tropical plant that is a perennial grass of *Graminaceae* family and genus *Cymbopogon*. It is a native plant with medicinal and fragrant characteristics. At local levels, it is identified with various names, such as 'Gawati Chah', 'Nibugrass', and 'Puthiganda'. In the subcontinent region, approximately three lemongrass species have been identified. The lemongrass that is grown in the eastern region of India is well known for its oil and has a growing market.[1] Lemongrass, a potential source of essential oils, is also a good potential source of phytochemicals that have pharmacological activities.[2]

As implied by its name, the plant is unique because it contains unstable oil that gives off a beautiful lemon scent. It is used as a healing ointment worldwide. Over 16,000 acres of lemongrass have been developed globally producing approximately 1,000 tonnes of lemongrass. Such production includes bulbous stems 100 cm long and 2 cm broad terete and glabrous leaf sheaths with a pointy edge, a narrow base, and a dramatic apex.[3,4] Several bioactive compounds can be found in the decoction, infusion, and essential oil isolates of lemongrass. Lemongrass tea has been shown to have antioxidant, anti-inflammatory, antibacterial, antiobesity, antinociceptive, anxiolytic, and other properties that support pharmacological claims about its properties. In several developing nations, alternative medicine practitioners widely embrace lemongrass tea because it is non-toxic, non-mutagenic, and safe.[5]

The aqueous extracts of lemongrass leaves possess hypoglycemic and hypolipidemic potential.[6] Lemongrass extracts have been reported to cure dermatitis. Depending on the age of the grass the composition of the oil changes. Fresh lemongrass produces 0.67% essential oil, which has a high citral content, while dry lemongrass produces 0.4% essential oil, which has a citral content of 72.3%.[7] Several studies confirmed that people of the subcontinent used lemongrass to cure headaches, nausea, colds, and rheumatic pain.[8] High concentrations of antimicrobial and antioxidant components are present in lemongrass extracts and oils.[9] Flavonoids, alkaloids, tannins, terpenes, phenolics, anthocyanins, steroids, saponins, isoflavones, coumarins, lignins, catechin, isocatechins, and ascorbic acid are among the many beneficial bioactive compounds present in lemongrass oil.[10]

From ancient times, herbal teas belonging to a range of scientific families, are typically used to cure fevers, hacking, colds, and as a pleasant tonic or beverage.[11] Herbal plants like lemongrass have been used to ease stomach pain, encourage perspiration, and produce mucus, as well as, being widely used for the treatment of gout.[12] Freshly cut and, to some extent, dried leaves of lemongrass are used for medical purposes and are the source of therapeutic oil. Many medicinal properties of C*ymbopogon citratus* include anti-amoebic, anti-bacterial, protection against intestinal looseness, anti-filarial, and anti-parasitic properties.[13] For different efficacy investigations to evaluate various health effects, Wang *et al.*[14] reported that extracts from lemongrass leaves have health-protective mechanisms.

Ethanolic and acetone extracts in polyphenols of lemongrass powder (LGP) had significantly higher antioxidant activities of their ethanolic extracts as compared to their acetone extracts.[15] Lemongrass leaves are a source of essential oils, which have an extraordinary role as natural preservatives due to their antimicrobial and antioxidant potential.[16] Due to their qualities as anticancer, antiviral, calming, anti-microbial, as well as, allopathic properties, phenolics and flavonoids are two types of polyphenols present in lemongrass, which have exhibited antioxidant and antimicrobial behavior in chicken sausages.[17]

As the world emerges from the destructive pandemic period, eating the right foods can help to develop and strengthen adaptive immunity. Plant-based foods, due to the presence of functional and nutraceutical components, are a useful part of a daily diet. In the post-COVID era, a sufficient supply of healthy foods that are balanced with pharmaceutical foods may play a fundamental role in boosting the immune systems of the populace.[18] As part of this functional food covenant, global communities are looking for fresh, nutritious food items that are rich in bioactive ingredients like fiber, minerals, vital amino acids, and phenols.[19] Worldwide consumption of bakery products like biscuits and cookies is high; however, such foods lack adequate nutritional value. To enhance bakery products with phytochemicals like phenolics, flavonoids, vitamins, minerals, carotenoids, natural colorants, and fiber, the substances from powdered plants, herbs, fruits as well as vegetables may be used as functional ingredients.[20] Different parts of plants may be extracted to obtain condensed phytochemicals, which exhibit strong antioxidant potential upon consumption.[21]

Despite the use of extracts, essential oils, and isolates from lemongrass for medicinal purposes, the scientific community has only started to realize the importance of pharma foods, such as those equipped with phytochemicals that are transferred from medicinal plants. For such purposes, recent advances have been made including, the manufacture of functional yoghurt with lemongrass extracts,[22] the development of lemongrass-flavored high-nutrient cookies,[23,24] and the production of lemongrass-incorporated meat sausages.[17] Compared with previous food product development, in current studies comparatively high levels of LGP were investigated for the development of nutritional biscuits, which were analyzed for phytochemical and sensory characteristics. To fulfil the need for healthy, pharma foods, the objectives of the current research were to convert lemongrass leaves into powder and then into ethanolic extracts for estimation of phytochemical composition. Such undertaking was done to create nutritional biscuits using different replacement levels of LGP and to analyze the phytochemicals, mineral profiles, and proximate composition of such biscuits. The evidence regarding the phytochemistry of lemongrass and the possible applications of LGP in food products were taken into consideration when completing the research.

Materials and methods

Collection of raw material

Fresh health leaves, uniform in size, were collected from the botanical garden of district Sargodha of province Punjab, Pakistan. The basis for raw material selection was color and physical status, *i.e.*, green color and healthy, disease-free leaves. After raw material collection, the specimen was submitted to the Department of Botany, University of Sargodha, for identification. For the biscuit preparation, ingredients were obtained from the resident market in district Sargodha. For each trial ingredients of the same brand were used.

LGP preparation

After the selection of healthy, disease-free, and matured leaves of lemongrass, the leaves were then subjected to washing (with clean water), cutting, blanching and then, subsequently, drying. The lemongrass leaves were dried using the cabinet dryer (TS-2266, Biobase, China), at 45 °C for almost 7 hours. Once the leaves were dried thoroughly, by following the methodologies of Thorat *et al.*,[24] they were ground into a fine powder by using a spice grinder (PS-110, Panasonic, Japan), sieved (80 mesh), and then, packed and stored for further analyses.

Preparation of lemongrass extracts

After Lemongrass (*Cymbopogon citratus*), was ground into a fine powder the instructions of Irfan *et al.*,[15] were followed for the preparation of ethanolic extracts. Briefly, the powder was extracted in 70% ethanolic solution with 30% water (1 g leaf powder with 20 mL ethanol and water solvent) overnight above room temperature. Via gravity filtration, the extracts were filtered using the P8 uneven filter, which was tailed by the vacuum clarification using a 0.45 µm filter. The extracts were dispersed at 40 °C in a rotary evaporator and again constituted in ethanol to obtain the final stock of 200 mg/mL concentration. The ethanolic extracts were then passed through an Acro disc of 0.2 µm DMSO-safe syringe filter in a biosafety filing cabinet.

Development of formulated biscuits with various replacement levels of LGP

The method used to make the biscuits by Hussain *et al.*[25] was modified in a few key areas. In brief, the ingredients were measured, combined, and sheeted to create the batter. With the aid of molds, biscuits were then shaped and set in the stainless-steel trays. The heating process took place in a baking oven set to 180 °C for 20 minutes. Wheat flour was used to make biscuits, with each replacement containing a varying amount of LGP. Control biscuits were developed with 100% wheat flour and in further treatments, wheat flour was replaced with LGP at 2.5, 5, 7.5, and 10% levels to assess the impact of LGP on the quality of biscuits.

Chemical analysis of LGP, wheat flour and developed biscuits

The chemical analysis of LGP, wheat flour, and developed biscuits was performed by following their respective protocols of methodologies.

Proximate analysis:

According to the procedures prescribed by the AAAC,[26] the proximate analyses of the LGP were carried out for ash, fat, fiber, moisture, and protein content, by following their respective methods. Such methods include the percentage of moisture method no. 44-15A, protein method no. 46-12, fat method no. 30-10, fiber method no. 32-10, and ash method no. 8-01 of the AACC[26] were all used, after required modifications. Similarly, the proximate analyses of wheat flour and LGP-incorporated biscuits were determined by adopting the same procedures, also with required modifications.

Mineral analysis

The analyses of essential minerals present in LGP, wheat flour, and formulated biscuits were carried out by using atomic absorption spectrophotometer (Perkin-Elmer Model 303, Canada), after the breakdown of samples with the acid mixture using air acetylene flame, by following the guidelines given by Alemayehu *et al.*[27] Briefly, a small quantity of the powder (1 g) sample was taken and then dissolved in the mixture of acids (nitric and perchloric acid) at 180–200 °C until the mixture solution of acids and powder turned clear. To make the final volume, 100 mL of double distilled water was added to the solution mixture. Using the atomic absorption spectrophotometer, the elemental analysis of the mixture solutions was performed. After performing each trial three times, mean values were determined.

Determination of total phenolic content (TPC)

Following the methodology as proposed by Shen *et al.*,[28] total phenolic contents in the ethanolic extracts of LGP, wheat flour, and developed biscuits were determined using a VIS-spectrophotometer (UV-1900i, Shimadzu, Japan), with some required modifications. In brief, 1 mL aliquots of the standard solution were mixed with 0.5 N Folin-Ciocalteu reagent (0.5 mL), and then by using 75 g/L sodium carbonate, the reaction was neutralized at 23 °C for approximately 2 hours. With the help of a gallic acid solution, a calibration curve was prepared. The phenolic contents were expressed as mg gallic acid equivalent per 100 g of dry weight.

Determination of total flavonoid content (TFC)

The colorimetric method used by Bao *et al.*[29] was followed for TFC determination, with some modifications. Explaining the protocols, aliquots (0.5 mL) of approximately diluted solution were taken in 15 mL polypropylene conical tubes that contained double distilled water (2 mL) and 5% $NaNO2$ (0.15 mL). Then, 1 mL of 1 M NaOH was added after 5 minutes. The reaction solution was thoroughly mixed and kept for another 15 minutes. Using the catechin curve the total flavonoid contents were determined by using VIS-spectrophotometer (UV-1900i, Shimadzu, Japan), in triplicate and expressed as mg of catechin equivalent per 100 g of dried weight.

Determination of total carotenoid content (TC)

For carotenoid determination, the spectrophotometric technique was used as used by de Carvalho *et al.,*[30] with some modifications. Powder samples of lemongrass, wheat flour, and different biscuits, 25 g each, were poured into acetone/n-hexane (80 mL, 1:1, vol/vol) and were thoroughly mixed and filtered under vacuum. The process was repeated several times until the samples were colorless. The organic phase was removed by using the separation technique to prevent emulsion formation. After the aqueous phase was discarded, the process was repeated many times until no residual solvent was left. With the help of anhydrous sodium sulfate organic dehydration phase was carried out. Carotenoid values were expressed as mg/100 g dried weight, and next, all readings were calculated in triplicate by using VIS-spectrophotometer (UV-1900i, Shimadzu, Japan), and the mean values were calculated.

Determination of DPPH free radical scavenging activity

DPPH free radical scavenging activity was determined using the standards illustrated by Brand Williams *et al.*,[31] with necessary modifications. DPPH of a small quantity of 0.01 g was poured into a 25 mL flask having methanol and water solution (80:20 vol/vol). An ascorbic acid calibration curve was also made. In microplates, 100 µl of each sample was taken and then 2 mL solvent and 250 µl DPPH were added. The whole mixture was shaken and mixed thoroughly and placed in the dark at ambient temperature for approximately 30 minutes. The results were expressed as mg of ascorbic acid equivalent/100 g powder for 30 minutes in the reaction after the mean value was computed for each sample.

Sensory evaluation of developed biscuits

The developed products that included LGP-incorporated biscuits, were sensory evaluated using a nine-point hedonic rating scale as outlined by Tsikritzi *et al.*[32] In a nutshell, a group of 20 specialists with an average age of 45 and representation from both genders were given sheets with ratings from 1 to 9, where 1 represented an extreme dislike and 9 represented an extreme like. The specialists were given sample biscuits with particular codes along with distilled water bottles for mouth washing and neutralizing after each test. The gathered information underwent calculation and analysis.

Ethics approval and consent to participate

This study does not involve any experiments on humans and animals. However, for sensory evaluation of developed products, as there are no mandatory national laws, appropriate protocols for protecting the rights and privacy of all participants were utilized during the execution of the research. Participants gave informed consent via the statement "I am aware that my responses are confidential, and I agree to participate in this survey" where an affirmative reply was required to enter the survey. They were able to withdraw from the survey at any time without giving a reason. The products tested were safe for consumption.

Table 1. Analysis of moisture, ash, protein, fat and fiber contents in wheat flour and LGP

Flours	Proximate analyses (%) of wheat flour and LGP				
	Moisture	Ash	Protein	Fat	Fiber
Wheat Flour	12.95 ± 0.04a	1.15 ± 0.01b	10.36 ± 0.06a	0.82 ± 0.02b	0.92 ± 0.04a
LGP	8.12 ± 0.02b	7.26 ± 0.06a	8.18 ± 0.04b	1.89 ± 0.04a	8.34 ± 0.04a

Column-wise values with similar alphabetical letters indicate non-significant results, while values with dissimilar alphabetic letters indicate significant results. LGP, lemongrass powder.

Table 2. Analysis of important minerals (calcium, magnesium, potassium, iron and zinc) present in wheat flour and LGP

Flours	Mineral composition (mg/100 g) of wheat flour and LGP				
	Calcium	Magnesium	Potassium	Iron	Zinc
Wheat Flour	30.44 ± 0.08b	42.98 ± 0.9b	38.68 ± 0.15b	2.75 ± 0.04b	2.52 ± 0.04b
LGP	36.80 ± 0.12a	64.89 ± 0.13a	54.65 ± 0.18a	12.68 ± 0.05a	8.46 ± 0.07a

Column-wise values with similar alphabetical letters indicate non-significant results, while values with dissimilar alphabetic letters indicate significant results. LGP, lemongrass powder.

Statistical analyses of the data

Results were reported as means ± standard deviations, and all analyses were carried out in triplicate to obtain determinations. The one-way ANOVA method was used for the statistical study. To distinguish between the mean numbers, Duncan's multiple-range test was utilized by following the protocols elaborated by Steel *et al.*[33]

Results

Proximate composition analyses of wheat flour and LGP

Results regarding the proximate composition of LGP and wheat flour have been shown in Table 1 that illustrates LGP contained higher contents of ash (7.26 ± 0.06%), fiber (8.34 ± 0.04%), and fat 1.89 ± 0.04%), whereas moisture and protein contents were greater in wheat flour.

Mineral composition of wheat flour and LGP

A comparison of important minerals present in wheat flour and LGP has been shown in Table 2, where it was evident that contents of Fe (12.68 ± 0.05 mg/100 g), Zn (8.46 ± 0.07 mg/100 g), Ca (36.80 ± 0.12 mg/100 g), Mg (64.89 ± 0.13 mg/100 g), and K (54.65 ± 0.18 mg/100 g) were significantly greater in LGP as compared to wheat flour, which opened the route of incorporation of LGP in wheat flour as an efficient source of minerals to meet the mineral deficiencies of bakery items.

TPC, TFC, TC and DPPH free radical scavenging activity of wheat flour and LGP

Results presented in Table 3 provided the values of TPC, TFC, TC, and DPPH free radical scavenging activities of wheat flour and LGP. From the results, it is evident that values of TPC, TFC, TC, and DPPH free radical scavenging activities of wheat flour were 50.30 mg GAE/100 g, 20.38 mg CE/100 g, 12.36 mg/100 g, and 24.36 mg AAE/100 g, respectively, whereas in LGP they were 240.46 mg, GAE/100 g, 98.45 mg CE/100 g, 62.36 mg/100 g, and 60.18 mg AAE/100 g, respectively. Comparing the bioactive compounds of wheat flour with those present in LGP, it can be concluded that LGP has many more phenolics, flavonoids, and carotenoids. Such high values of bioactive compounds found in extracts of lemongrass have been associated with the medicinal potential of lemongrass as phenolics, flavonoids, and carotenoids have strong antioxidant and antimicrobial activities.

Proximate composition of LGP- incorporated biscuits

Table 4 presents the results for moisture, ash, fat, fiber, and protein contents, both in control biscuits and the LPG-developed biscuits. From the results, it was clear that the incorporation of LGP increased ash, fat, and fiber contents of formulated biscuits, whereas protein and moisture contents were decreased. Contents of ash and fiber in control biscuits were 1.89 ± 0.01 and 0.43 ± 0.01%, respectively, which were significantly increased to 3.10 ± 0.04 and 3.25 ± 0.06% respectively in 10% LGP-incorporated biscuits, which occurred due to higher ash and fiber contents present in LGP. The decrease in moisture and protein contents was due to lower moisture and protein contents in LGP, as compared to wheat flour.

Mineral composition of LGP- incorporated biscuits

Results of mineral contents present in LGP-incorporated biscuits have been presented in Table 5, from where it was clear that the fortification of LGP in different ratios significantly increased the mineral contents of fortified biscuits. Values of Ca, Mg, K, Fe, and

Table 3. Total phenolic contents, total flavonoid contents, total carotenoids and DPPH free radical scavenging activity of wheat flour and LGP

Flours	Total Phenolic Content (mg GAE/100 g)	Total Flavonoid Content (mg CE/100 g)	Total Carotenoids (mg/100 g)	DPPH free radical scavenging activity (mg AAE/100 g)
Wheat Flour	50.30 ± 0.13b	20.38 ± 0.08b	12.36 ± 0.14b	24.36 ± 0.09b
LGP	240.46 ± 0.20a	98.45 ± 0.15a	62.36 ± 0.12a	60.18 ± 0.14a

Column-wise values with similar alphabetical letters indicate non-significant results, while values with dissimilar alphabetic letters indicate significant results. AAE, ascorbic acid equivalent; CE, catechin equivalent; DPPH, 2,2-diphenyl-1-picrylhydrazyl; GAE, gallic acid equivalent; LGP, lemongrass powder.

Table 4. Analysis of moisture, ash, protein, fat and fiber contents in LGP-incorporated biscuits

Substitution Plan of Biscuits	Proximate composition (%) of LGP-incorporated biscuits				
	Moisture	Ash	Protein	Fat	Fiber
0% (Control)	5.36 ± 0.04a	1.89 ± 0.01e	7.28 ± 0.04a	26.32 ± 0.05c	0.43 ± 0.01e
2.5% LGP	5.12 ± 0.02b	2.06 ± 0.01d	7.20 ± 0.02a	26.75 ± 0.08b	0.89 ± 0.03d
5% LGP	4.92 ± 0.01c	2.46 ± 0.01c	7.02 ± 0.01ab	26.97 ± 0.02bc	1.56 ± 0.02c
7.5% LGP	4.50 ± 0.02d	2.83 ± 0.01b	6.92 ± 0.02ab	27.15 ± 0.07ab	2.48 ± 0.03b
10% LGP	4.26 ± 0.03e	3.10 ± 0.04a	6.62 ± 0.03c	27.62 ± 0.04a	3.25 ± 0.06a

Column-wise values with similar alphabetical letters indicate non-significant results, while values with dissimilar alphabetic letters indicate significant results. LGP, lemongrass powder.

Table 5. Analysis of important minerals (calcium, magnesium, potassium, iron, and zinc) present in LGP-incorporated biscuits

Substitution Plan of Biscuits	Mineral Composition (mg/100 g) of LGP-incorporated biscuits				
	Calcium	Magnesium	Potassium	Iron	Zinc
0% (Control)	22.46 ± 0.16e	15.68 ± 0.09e	49.23 ± 0.09e	2.08 ± 0.02e	1.18 ± 0.02e
2.5% LGP	23.39 ± 0.12d	18.45 ± 0.10d	51.73 ± 0.14d	3.14 ± 0.07d	1.92 ± 0.04d
5% LGP	23.98 ± 0.09c	20.45 ± 0.11c	52.82 ± 0.12c	4.42 ± 0.08c	2.69 ± 0.04c
7.5% LGP	24.76 ± 0.08b	23.76 ± 0.12b	54.10 ± 0.12b	5.84 ± 0.03b	3.56 ± 0.05b
10% LGP	25.50 ± 0.04a	26.83 ± 0.16a	56.26 ± 0.18a	7.02 ± 0.04a	4.27 ± 0.02a

Column-wise values with similar alphabetical letters indicate non-significant results, while values with dissimilar alphabetic letters indicate significant results. LGP, lemongrass powder.

Zn in control biscuits were 22.46 ± 0.16, 15.68 ± 0.09, 49.23 ± 0.09, 2.08 ± 0.02, and 1.18 ± 0.02 mg/100 g biscuits, respectively, which were significantly higher in 10% LGP incorporated biscuits at 25.50 ± 0.04, 26.83 ± 0.16, 56.26 ± 0.18, 7.02 ± 0.04 and 4.27 ± 0.02 mg/100 g biscuits, respectively. Significantly high amounts of minerals, especially Zn and Fe in 10% LGP incorporated biscuits, were aspects of this study, to be taken as positive for the development of pharma foods. The provision of zinc and iron to the malnourished populations, a challenging task, could be accomplished through the development, marketing and consumption of LGP-incorporated biscuits.

TPC, TFC and total carotenoids and DPPH free radical scavenging activity of LGP-incorporated biscuits

Results presented in Table 6 provide the TPC, TFC, TC and DPPH free radical scavenging activity of control and LGP incorporated biscuits. Amounts of TPC, TFC, TC and DPPH free radical scavenging activities of control biscuits were significantly lower than those of 10% LGP incorporated biscuits and the values were 50.30 mg GAE/100 g, 20.38 mg CE/100 g, 1.96 mg/100 g, and 5.88 mg AAE/100 g, respectively. On the other hand, the values were significantly boosted in 10% LGP incorporated biscuits and were 260.45 mg GAE/100 g, 62.80 mg CE/100 g, 5.03 mg/100 g and 17.42 mg AAE/100 g, respectively. From the experimental values, a positive correlation can be observed among phenolics, flavonoids, carotenoids, and antioxidant activity, as an increment of the bioactive compounds caused an increment in the antioxidant capacity of biscuits.

Sensory evaluation of LGP-incorporated biscuits

Results containing scores of sensory evaluation of different treatment biscuits have been shown in Table 7, from where it can be easily analyzed that for color and flavor of biscuits, a 5% replacement level of LGP was preferred by judges, whereas a 2.5% level of LGP got best scores for texture, taste and overall acceptability. On the other hand, higher replacement levels, although improving

Table 6. Total phenolic contents, total flavonoid contents, total carotenoids and DPPH free radical scavenging activity of LGP- incorporated biscuits

Substitution Plan of Biscuits	Total Phenolic Contents (mg GAE/100 g)	Total Flavonoid Contents (mg CE/100 g)	Total Carotenoids (mg/100 g)	DPPH free radical scavenging activity (mg AAE/100 g)
0% (Control)	50.30 ± 0.12e	20.38 ± 0.04e	1.96 ± 0.03e	5.88 ± 0.02e
2.5% LGP	140.46 ± 0.10d	38.45 ± 0.02d	3.58 ± 0.02d	8.20 ± 0.02d
5% LGP	155.78 ± 0.17c	46.35 ± 0.04c	3.96 ± 0.02c	10.79 ± 0.03c
7.5% LGP	210.30 ± 0.13b	53.89 ± 0.03b	4.28 ± 0.02b	14.25 ± 0.02b
10% LGP	260.45 ± 0.13a	62.80 ± 0.09a	5.03 ± 0.04a	17.42 ± 0.03a

Column-wise values with similar alphabetical letters indicate non-significant results, while values with dissimilar alphabetic letters indicate significant results. AAE, ascorbic acid equivalent; CE, catechin equivalent; DPPH, 2,2-diphenyl-1-picrylhydrazyl; GAE, gallic acid equivalent; LGP, lemongrass powder.

Table 7. Sensory evaluation of LGP- incorporated biscuits

Substitution Plan of Biscuits	Sensory evaluation parameters LGP-incorporated biscuits				
	Color	Flavor	Taste	Texture	Overall acceptability
0% (Control)	7.30 ± 0.70b	7.10 ± 0.71b	7.40 ± 0.70b	7.25 ± 0.67b	7.40 ± 0.70b
2.5% LGP	7.60 ± 0.52a	7.80 ± 0.52a	7.80 ± 0.71a	7.65 ± 0.52a	7.60 ± 0.52a
5% LGP	7.62 ± 0.52a	6.75 ± 0.52a	7.20 ± 0.52c	6.78 ± 0.70c	7.36 ± 0.48b
7.5% LGP	6.38 ± 0.52c	7.20 ± 0.63b	6.40 ± 0.63d	6.32 ± 0.48d	5.85 ± 0.48c
10% LGP	5.20 ± 0.52 d	6.22 ± 0.63c	5.50 ± 0.63e	5.25 ± 0.48e	5.42 ± 0.48d

Column-wise values with similar alphabetical letters indicate non-significant results, while values with dissimilar alphabetic letters indicate significant results. LGP, lemongrass powder.

the nutritional values of the developed biscuits, had their sensory scores significantly decreased for all parameters. Overall acceptability scores were just 5.42 ± 0.48 for 10% LGP incorporated biscuits, which were significantly lesser than 7.6 ± 0.52, the scores of 2.5% LGP incorporated biscuits. The optimum replacement level of LGP to develop good quality biscuits, which were not only of nutritional and medicinal importance but also liked well by consumers, a 2.5 to 5% level of replacement could be recommended that might prove effective for both food producers and consumers alike.

Discussion

Higher contents of ash and fiber were positive indications for utilizing LGP as a source of functional components in food products. Radwan and Elmaadawy[34] provided the values of ash, fat, fiber, protein, and moisture contents of LGP, which did not differ significantly from those currently documented. Hussain *et al.*[25] performed a proximate analysis of white flour before the development of nutritional biscuits and provided values of moisture, ash, fat, fiber, and protein contents in white flour as 13.56, 1.05, 0.97, 0.72, and 10.11 mg/100 g, respectively. Such values were closely related to the results of the present study where the authors identified lesser amounts of ash, fat and fiber contents in wheat flour as compared to LGP. Figure 1 presents the summarized work plan conducted on lemongrass and lemongrass powder incorporated biscuits.

Thorat *et al.*[24] produced LGP and determined proximate composition of lemongrass fresh leaves and then developed powder, with values of moisture, ash, fat, protein, and carbohydrates recorded at 7.01, 11.28, 1.45, 11.15, and 65.78%, respectively. Reported higher values of ash and protein may be due to cultivation and climate differences of the lemongrass that affect the chemical composition among the same varieties of the same crops. From the results of Thorat *et al.*,[24] it was evident that there is a decrease in moisture, protein, and fat content and an increase in carbohydrates, fiber, and ash content of wheat flour-based bakery products that occurred due to the specific proximate composition of LGP. Similar results for proximate analysis of LGP were also present in the research work of Irfan *et al.*,[15] highlighting the higher contents of ash and fiber.

Performing similar experiments on lemongrass, Asaolu *et al.*[35] reported moisture, protein, ash, and fiber contents in LGP as 5.76, 4.56, 20.30, and 55%, respectively. Higher values of fiber and ash contents in their study might be due to environmental and agricultural land factors. Alemayehu *et al.*[27] evaluated another medicinal plant, nettle leaves (*Urtica simensis*) flour for proximate composition and results revealed that this plant contained a sufficient amount of ash, protein, and fiber, to be utilized in wheat flour for the development of noodles, and this can be connected with the present research, as use of medicinal plants powders could prove helpful in the development of pharma foods.

Birhanu *et al.*[36] determined some important minerals present in lemongrass and the results supported our study. Significant amounts of iron, zinc, magnesium, calcium, and potassium were found in lemongrass. According to the results, lemongrasses are an important origin of essential minerals. The mineral concentrations in the examined samples fell under WHO's maximum allowable limits, making them safe for consumption by people. Their data reflected the average content of calcium, magnesium, potassium, iron, and zinc in LGP, which were closely related to the present findings. Giving findings just in line with current ones, Ranade and Thiagarajan[37] reported that LGP contained high calcium, phosphorus, and potassium values. The deficiency of wheat flour for zinc and iron was reported by Hussain *et al.*[25] when they provided the respective values of iron and zinc as 2.85 and 2.63 mg/100 g of white flour, which was closely related to the findings given in the current work, and these values were much less than present in LGP.

Several earlier investigations have provided strong scientific evidence about the presence of both trace and macro minerals in wild and domestic medicinal plants, due to which these plants and their powders find their route into pharma foods. Asaolu *et al.*[35] explored some macro and trace minerals in LGP and found sufficient quantities of Se, Zn, Fe, Ca, Cu, and K. They reported slightly higher values of Fe and Zn in LGP as compared to current results, and such variation in results might be due to several factors including maturity stage of the plant used, cultivation environment, genotype, and techniques adopted for analysis. While performing similar research work, Alemayehu *et al.*[27] explored mineral contents in a wild medicinal plant powder, before its utilization in extruded product. They reported that nettle leaves (*Urtica simensis*) flour possessed sufficient amounts of iron, zinc, and calcium.

Phenolics, flavonoids, and carotenoids are among the important phytochemical classes, known for their health-promoting potential, as they stop the onset of many diseases. Bioactive substances are essential for human nutrition and health.[38] In greater amounts, the antimicrobial and antioxidants are present in lemongrass oil. Extracts and oils of lemongrass, both rich in several beneficial bioactive substances other than phenolics and flavonoids, which are alkaloids, tannins, anthocyanins, steroids, terpenes, saponins, isoflavones, coumarins, lignins, catechin, isocatechins, as well as, ascorbic acid, and all such components have their positive role in scavenging free radicals thus acting as antioxidants.[9,10] Thorat *et al.*[24] analyzed phenolic and flavonoid components in lemongrass

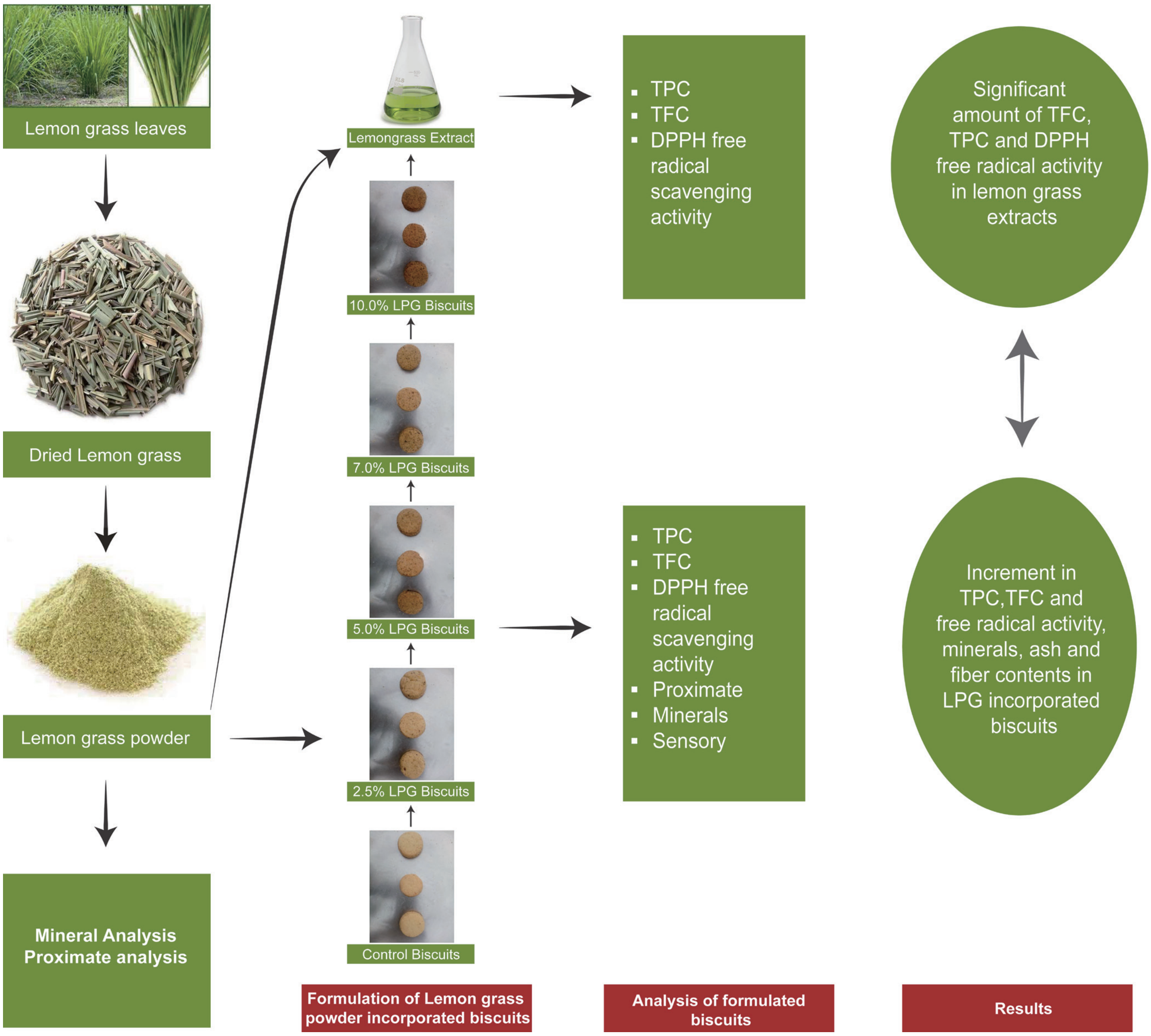

Fig. 1. Summarized work plan, conducted on lemongrass and lemongrass powder incorporated biscuits.

extracts and the results did not differ greatly from this study. Similar findings have also been documented in the experiments of Irfan *et al.*,[15] as they used acetone and ethanol as solvents for extraction of LGP and observed higher contents of TPC and TFC in ethanolic extracts with higher antioxidant activity, strengthening the use of ethanol as an effective solvent for maximizing phytochemicals from LGP. Falah *et al.*[39] reported that the extract of lemongrass in 70% ethanol had the lowest IC_{50} value (79.4) and the highest DPPH inhibition, which displayed the strong antioxidant potential of this important herb, just as was proven by our experiments.

In another similar study, Asaolu *et al.*[35] investigated lemongrass extracts for the presence of phytochemicals and found appreciable values of phenolics, flavonoids, alkaloids, saponins, tannins, and steroids, which have a strong relationship with health benefits. Further, they reported that extracts of lemongrass exhibited strong antibacterial activities also, against selected bacterial species. Relevant findings were also seen when Lu *et al.*[9] used three different methods to determine the antioxidant activity of lemongrass extracts and reported that lemongrass extracts possess significant antioxidant activity very compatible with ascorbic acid, gallic acid, and quercetin. As in the current experiment phenolics, flavonoids, and antioxidant activity were calculated in equivalence to the same standards. Polyphenols, which are renowned for their anti-inflammatory and antioxidant properties, are abundant in lemongrass. In an experiment exploring lemongrass oils and extracts, an aqueous residue was produced after hydro-distilling lemongrass essential oil and was found to have a similar phenolic profile as lemongrass extracts and oils. Aqueous waste and extracts of lemongrass, both exhibited strong DPPH free radical scavenging and anti-inflammatory activities in safe modes, without any toxicity,[10] which proved that ethanolic extracts, as well as, aqueous extracts of lemongrass have sufficient bioactive compounds to be utilized as medicinal agents.

The medicinal importance of lemongrass extracts has been widely recognized around the globe. Lemongrass extracts, when applied to animals, exhibited hypoglycemic and hypolipidemic activities, which might be attributed to the bioactive components present in this medicinal herb.[6] Boeira *et al.*[17] extracted bioactive components from lemongrass and incorporated them in meat products to check their antimicrobial and antioxidant activities. They stated that phenolics and flavonoids are the major bioactive compounds responsible for such medicinal properties. To reduce the risk of drug-related toxicity and improve the effectiveness of the therapy, lemongrass extracts may be used in conjunction with chemotherapeutics. Lemongrass extract has been shown in a clinical trial to have selective anticancer activity.[40] Mohamed *et al.*[41] proved that as compared to the gentamicin group alone, the rats treated with LGP and gentamicin exhibited substantial alterations in blood urea, nitrogen, and creatinine. During the blood biochemical studies, the use of lemongrass extract to treat the liver and kidney tissues improved the health of rats. Such findings can be related to the presence of phenolics and flavonoids, which have antioxidant potential in animals.

In a food material ash content is the non-organic compounds in the food that contain mineral content. Nutritionally, it helps in the process of metabolism of other organic compounds such as carbohydrates and fats.[42] Kukade *et al.*[23] performed a similar experiment by developing lemongrass-flavored high-nutrient cookies. Three cookies were prepared by the substitution of wheat flour with poppy seed flour and LGP in different ratios and then were tasted for moisture, ash, protein and crude fiber, *etc.* The results showed that calorific values like fat, moisture, and fiber, among others were increased by using enriched wheat flour in place of refined wheat flour. Outcomes from the study of Radwan and Elmaadawy[34] were in line with current results where investigators developed nutritional bread by incorporating LGP at various levels to find a remarkable increase in ash and fiber contents of the developed bread. Ash and fiber contents in control bread were 1.20 and 5.72% respectively, which were increased to 1.48 and 6.34% in bread developed with 10% LGP. Supportive findings were also in the studies of Thorat *et al.*[24] when they incorporated LGP in cookies at different levels and observed increases in ash, fat, and fiber contents of cookies. The increment of ash and fiber contents of biscuits developed as a result of the incorporation of lemon pomace powder was recently reported in the study of Hussain *et al.*[43]

The development of herbal cookies by Yadav *et al.*[44] also indicated that the addition of organic herbs reduced the moisture contents in the developed cookies. The low contents of moisture in supplement cookies were because of the low water absorbability and low water contents of incorporated herbs, which also support our research, as the addition of LGP into the developed biscuits reduced the moisture contents in LGP fortified cookies. Similar results obtained from the work of Dias *et al.*,[45] can also be used here to support our findings as they performed the nutritional assessment of composite cookies of wheat flour incorporated with herbal plant powder. The level of ash, fat, protein, carbohydrate, and fiber of the herbal powder incorporated biscuits was in the range of USDA National Nutrient Database for Standard Reference. This research was also an indication that the fortification of lemongrass increases the ash, fat, and fiber contents.

Development of some other food products by incorporation of herbal plants powders was also observed in some studies, as Alemayehu *et al.*[27] incorporated wheat flour with dried nettle leaves (*Urtica simensis*) flour to develop noodles and noticed a noteworthy increase in moisture, ash, protein, and fiber contents of formulated noodles, whereas carbohydrates and fat contents were found in decreasing order. Their results supported the findings of the present research, that wild and medicinal plant powders can be used as nutritional ingredients in bakery and extruded snack products.

Minerals are essential for human nutrition to regulate various metabolic processes in the body and also provide support to the skeleton. Higher consumption of cereal-based bakery items has led to the deficiencies of important minerals in humans, which can be encountered by the incorporation of non-wheat flour from plants, fruits, and vegetables into wheat flour for the development of nutritional food products.[46] The high mineral contents of lemongrass are the foundation for the rise in calcium, iron, zinc, and other minerals in incorporated biscuits. Infants and young children need calcium for healthy growth and development, while iron and zinc are essential for metabolism and immunity. Magnesium is another mineral that is abundant in carrots. Magnesium is necessary for the synthesis of bone, protein, and new cells, the activation of B vitamins, the relaxation of muscles and nerves, blood coagulation, as well as many other human metabolic processes.[47]

Birhanu *et al.*[36] experimented to study the levels of trace and major minerals in lemongrass. After analysis of lemongrass through wet acid digestion, it was evaluated for minerals, utilizing the recovery test. Lemongrass was stated as the efficient source of minerals as the recovery percentage ranges from 86.9% to 106% and was all obtained from identified metals. The concentrations of metals K, Ca, Mg, Fe, Mn, Cu, Zn, and Pb range from 743.8 to 1,020, 123.1 to 129.3, 23.9 to 36.3, 10.35 to 22.3, 10.0 to 12.7, 1.48 to 2.53, and 0.59 to 1.07 mg/ kg, respectively. The results of this research showed that lemongrass was a beneficial and efficient source of essential metals, and variations in quantities of minerals are dependent upon several factors like cultivar, genotype, maturity, purity of chemical used and techniques adopted for analysis. Similar, increments in the mineral contents can also be taken from the work of Bolarinwa *et al.*,[48] in which nutritional values and acceptability of the bread fortified with moringa were determined. Their study reported that the fortification of organic herbal plant powders significantly increased the mineral contents of wheat bread. Similar research was conducted by Boriy *et al.*[19] when herbal extracts fortified pan bread were developed, and the results of this finding were quite supportive of our work, as the chia seeds fortified bread had high values of minerals like magnesium, iron, zinc, and potassium.

Agrahar-Murugkar,[49] created fortified bread and biscuits by substituting refined wheat flour with herbage, African millet, seed, and spices, for enhancing the bio-accessibility and improvement of Ca, Zn, and Fe, among others. Their study's findings demonstrated the effectiveness of fortified bread and biscuits as a source of bioaccessible minerals and their role in enhancing dietary mineral status. Another example of the incorporation of herbal plant powders for the development of nutritional food items was observed when Alemayehu *et al.*[27] dried nettle leaves (*Urtica simensis*) to develop nutritional flour and incorporated in wheat flour for the development of noodles. By comparing the formulated noodles with the control sample, they concluded that a significant increase in essential minerals like calcium, iron, and zinc was observed in noodles, which was credited to the high mineral contents in the powder of the medicinal plant used. The observations of Hussain *et al.*[25] also produced similar outcomes to those herein, as they reported an increase in iron and zinc contents of wheat biscuits when incorporated with different portions of pumpkin powder.

Lemongrass methanolic extracts were involved in antioxidant and antimicrobial activities, which was probably due to their high

TPC and TFC values.[50] In another study, three different varieties of lemongrass were tested for their phytochemical and antioxidant potential, and the results were promising, indicating the directly proportional relation between TPC and DPPH free radical scavenging activities of lemongrass extracts.[51] Revealing the medicinal importance of this medicinal herb, fresh and dried lemongrass leaves were compared with commercially available teas for the presence of phytochemicals and antioxidant activities, and results showed that this herbal plant has sufficient amounts of TFC and TPC with high antioxidant capacities.[52] The antioxidant effect of lemongrass was tested in another study, when lemongrass oils were introduced in soya bean oil, and DPPH assay was performed to calculate free radical scavenging activity. Further, the TPC and TFC of formulated oils were also higher.[53]

While baking cookies, made with the incorporation of different nutritional flours with high TPC and TFC values, it was observed that baking did not affect the TPC of the flour or dough, but slightly decreased the level of TFC, nonetheless benefitting consumers by increasing nutrition through the incorporation of different plant flours in cookies. In addition, it was shown that the developed cookies contained high phytochemicals.[54] Another study by Dias *et al.*[45] reported the presence of phytochemicals including flavonoids, phenolics, alkaloids, saponins and tannins in herbal cookies, developed to promote human health.

Polyphenols are the most prevalent and easily available phenolic contents in plants, and according to Valenzuela *et al.*,[55] plants contain large amounts of polyphenol compounds, which give them many of their antioxidant qualities. The amount of DPPH free radicals that can be scavenged is directly proportional to the polyphenol content. *Euphorbia eriophora*, a medicinal plant, was shown to have DPPH free radical scavenging activity, and it was established that a lot of products can benefit from using this native plant as a natural antioxidant agent.[56]

Many examples from earlier studies showed that adding plant powders to biscuits increased their TPC equated to normal wheat flour biscuits. In a study, Ismail *et al.*[57] measured the TPC and DPPH free radical scavenging activities of the developed cookies by adding pomegranate peel powder in various ratios to straight-grade flour. Cookies made with 100% straight-grade flour had a TPC of 90.70 mg GAE/100 g, and as the replacement amount of pomegranate peel flour was raised, a discernible rise in the TPC of cookies was observed. By adding pumpkin peel, flesh, and seeds powders in various quantities to nutritional biscuits, Hussain *et al.*[58] achieved results that were remarkably similar to those of the current study in terms of the biscuits' capacity to scavenge free radicals, as TPC, TFC, TC, and DPPH activity were raised. Choi[59] made pine needle cookies that were shown to have a modest DPPH activity (6%) when made with 100% wheat flour, but a large increase (60%) was seen when up to 5% pine needle flour was added. Also, he concluded that cookies with higher total phenolic contents exhibit greater antioxidant activity.

The sensory properties of the herbal biscuits incorporated with LGP, including color, flavor, taste, texture, and overall acceptability, were evaluated using a 9-point hedonic scale. Increased LGP content over 3% has a negative impact on the sensory quality of cooked cookies, as was reported by Thorat *et al.*,[24] after an experimental study, whereas our experiments concluded that up to 5% level could be acceptable for the development of good quality biscuits. Supportive findings were also present in the trials of Hussain *et al.*[25] observing the use of fruits and vegetable powders at lower levels proved beneficial to develop acceptable bakery products. The current study's findings were also supported by the sensory analysis of a study by Bolarinwa *et al.*,[48] which showed that the bread made with 5% moringa was not significantly different from the bread made with 100% wheat flour, in terms of the majority of the quality attributes assessed. Similar findings were also present in the research work by Boriy *et al.*[19] during the development of pan breads, as suitable levels of incorporated powders can provide the required quality of bread.

Similarly, the sensory attributes of lemongrass and lime juice incorporated beverages developed by Kieling and Prudencio[60] were significantly positive on the 9-point hedonic scale. The results derived from the acceptance levels, based upon the characteristics of color, flavor, and aroma, among others, of that study suggested the high-level possibilities of incorporation of lemongrass constitutes in various food commodities would possibly bring positive acceptance outcomes. This would also help the consumers to attain better health and nutrition status by utilizing the properties of various constituents present in these fortified commodities. Findings of a recent study by Hussain *et al.*[43] also witnessed that suitable replacement levels of flour from lemon pomace provided optimum quality biscuits with high nutritional components.

According to Abdel-Naeem *et al.*,[61] using powders from lemon, orange, grapefruit, and banana peels increased the organoleptic scores for chicken patties in terms of color, aroma, appearance, and tenderness. The developed final products were well received by the judges, opening up new avenues for the development of dairy, bakery, beverage, and meat products by using non-wheat powders from medicinal plants. Similar results were also present in the studies of Alemayehu *et al.*[27] when they developed noodles with different formulations of wheat flour and a medicinal plant powder. They reported that a 5% incorporation level was found to be acceptable as this product produced good scores, close to the control. The presence of more flavoring compounds and polyphenols in LGP may account for the decreased flavor and taste scores of biscuits with 7.5 and 10% LGP replacement, while the presence of more fiber may also contribute to the decreased texture scores with higher LGP levels. Low scores of overall acceptability and appearance are a result of the 10% LGP biscuits' more yellow to greenish hue, which is a result of natural pigments found in lemongrass.

Lemongrass is a safe food additive with no toxicity

Lemongrass is believed to be harmless for both human health and the environment due to its non-toxic mechanism of action. The majority of non-target animals, including humans, are not poisoned by LGP or extracts, a common culinary ingredient in many cuisines. Lemongrass extracts are GRAS (Generally Recognized as Safe) food additives. The European Chemical Agency (ECHA) has recognized lemongrass oil as a chemical under the REACH (Registration, Evaluation, Authorization and Restriction of Chemicals) regulation. Lemongrass extracts guard food's sensory qualities and suppress microbial activity, avoiding food deterioration, maintaining product quality, and increasing shelf life.[16] In Pakistan there is no legal approval required to use lemongrass as a food additive, and from the literature reviewed, no toxicity effects of the use of lemongrass were observed. As lemongrass leaves used in the current study were manually harvested from the botanical garden of district Sargodha, Pakistan, and no use of any pesticide on lemongrass was confirmed by the management of the garden.

Conclusion

The predominance of phytochemicals in lemongrass draws scientists and researchers to use this cost-effective and nutritious herb for the development of functional foods. Lemongrass contains beneficial components that can be extracted, separated, and used in the manufacturing of medicines. In current research work, lemongrass leaves were converted into powders, which were further extracted using 80% ethanol to find out the presence of phytochemicals in this herb. Concluding the major findings, significantly higher amounts of ash, fiber, Zn, Fe, Ca, Mg and K were present in LGP, as compared to wheat flour, which resulted in an increment of these parameters in developed biscuits. Results revealed that lemongrass extracts were rich in polyphenols, especially phenolics and flavonoids, which have strong antioxidant properties. Phytochemical analyses of extracts provided values of TPC 240.46 ± 0.20 mg GAE/100 g, TFC 98.45 ± 0.15 mg CE/100 g, TC 62.36 ± 0.12 mg/100 g and DPPH free radical scavenging activity as 60.18 ± 0.14 mg AAE/100 g. During the development of LGP-incorporated biscuits, it was observed that as the level of LGP was increased in biscuits, values of TPC, TFC, TC, minerals, and DPPH free radical scavenging activity were also increased. Sensory panel experts preferred a 2.5% level of LGP as optimum, for good texture, taste and overall acceptability, whereas a 5% level was recommended best for color and flavor of the biscuits. In the end, it could be concluded that a 2.5 to 5% replacement level is suitable for developing good quality and acceptable biscuits, which could be consumed as pharma foods, due to the presence of high amounts of phytochemicals. Further, *in vivo* and *in vitro* trials with LGP-incorporated biscuits are recommended to find the health-promoting and disease-lowering capacity of lemongrass.

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Conflict of interest

The authors have declared no conflict of interests in this article.

Author contributions

Conceptualization and draft of the manuscript (AH); data curation, supervision, validation and visualization (TK); formal analysis (AB); funding acquisition and investigations (TMM, MS and HF); methodology (QUA); project administration (SY); resources (FIG); software (AR); review and editing (AH and MRA).

Ethics approval and consent to participate

This study does not involve any experiments on humans and animals. However, for sensory evaluation of developed products, as there are no mandatory national laws, appropriate protocols for protecting the rights and privacy of all participants were utilized during the execution of the research. Participants gave informed consent via the statement "I am aware that my responses are confidential, and I agree to participate in this survey" where an affirmative reply was required to enter the survey. They were able to withdraw from the survey at any time without giving a reason. The products tested were safe for consumption.

Data availability

Data relevant to this study can be provided upon request.

References

[1] Mujaffar S, John S. Thin-layer drying behavior of West Indian lemongrass (Cymbopogan citratus) leaves. Food Sci Nutr 2018;6(4):1085–1099. doi:10.1002/fsn3.642, PMID:29983973.

[2] Kumari P, Seth R, Meena A, Sharma D. Enzymatic synthesis of cellulose nanocrystals from lemongrass and its application in improving anti-cancer drug release, uptake and efficacy. Ind Crop Prod 2023;192:115933. doi:10.1016/j.indcrop.2022.115933.

[3] Saleem M, Afza N, Anwar MA, Hai SM, Ali MS. A comparative study of essential oils of Cymbopogon citratus and some members of the genus Citrus. Nat Prod Res 2003;17(5):369–373. doi:10.1080/14786410310001605823, PMID:14526919.

[4] Haque ANMA, Remadevi R, Naebe M. Lemongrass (Cymbopogon): a review on its structure, properties, applications and recent developments. Cellulose 2018;25:5455–5477. doi:10.1007/s10570-018-1965-2.

[5] Olorunnisola SK, Hammed AM, Simsek S. Biological properties of lemongrass: An overview. Int Food Res J 2014;21(2):455–462.

[6] Adeneye AA, Agbaje EO. Hypoglycemic and hypolipidemic effects of fresh leaf aqueous extract of Cymbopogon citratus Stapf. in rats. J Ethnopharmacol 2007;112(3):440–444. doi:10.1016/j.jep.2007.03.034, PMID:17513076.

[7] Bleasel N, Tate B, Rademaker M. Allergic contact dermatitis following exposure to essential oils. Australas J Dermatol 2002;43(3):211–213. doi:10.1046/j.1440-0960.2002.00598.x, PMID:12121401.

[8] Poddar S, Sarkar T, Choudhury S, Chatterjee S, Ghosh P. Indian traditional medicinal plants: A concise review. Int J Bot Studies 2023;5(5):174–190.

[9] Lu Y, Khoo TJ, Wiart C. Antioxidant activity determination of citronellal and crude extracts of Cymbopogon citratus by 3 different methods. Pharm Pharmacy 2014;5(4):395–400. doi:10.4236/pp.2014.54047.

[10] Tavares F, Costa G, Francisco V, Liberal J, Figueirinha A, Lopes MC, *et al*. Cymbopogon citratus industrial waste as a potential source of bioactive compounds. J Sci Food Agric 2015;95(13):2652–2659. doi:10.1002/jsfa.6999, PMID:25389117.

[11] Van Wyk BE, Gorelik B. The history and ethnobotany of Cape herbal teas. S Afr J Bot 2017;110:18–38. doi:10.1016/j.sajb.2016.11.011.

[12] Periyasamy S, Chandrasekar S, Pichaivel M, Velmurugan T. A write up on allopathic & herbal medicine for treatment of gout. Int J Herb Med 2020;8(5):14–22.

[13] Manvitha K, Bidya B. Review on pharmacological activity of Cymbopogon citratus. Int J Herb Med 2014;6:7.

[14] Wang L, Waltenberger B, Pferschy-Wenzig EM, Blunder M, Liu X, Malainer C, *et al*. Natural product agonists of peroxisome proliferator-activated receptor gamma (PPARγ): a review. Biochem Pharmacol 2014;92(1):73–89. doi:10.1016/j.bcp.2014.07.018, PMID:25083916.

[15] Irfan S, Ranjha MMAN, Nadeem M, Safdar MN, Jabbar S, Mahmood S, *et al*. Antioxidant activity and phenolic content of sonication-and maceration-assisted ethanol and acetone extracts of Cymbopogon citratus leaves. Separations 2022;9(9):244. doi:10.3390/separations9090244.

[16] Faheem F, Liu ZW, Rabail R, Haq IU, Gul M, Bryła M, *et al*. Uncovering the Industrial Potentials of Lemongrass Essential Oil as a Food Preservative: A Review. Antioxidants (Basel) 2022;11(4):720. doi:10.3390/antiox11040720, PMID:35453405.

[17] Hussain A, Kausar T, Sehar S, Sarwar A, Quddoos MY, Aslam J, *et al*. A review on biochemical constituents of pumpkin and their role as pharma foods; a key strategy to improve health in post COVID 19 period. Food Production, Processing and Nutrition 2023;5(1):1–14. doi:10.1186/s43014-023-00138-z.
[18] Boeira CP, Piovesan N, Soquetta MB, Flores DCB, Lucas BN, Rosa CSD, *et al*. Extraction of bioactive compounds of lemongrass, antioxidant activity and evaluation of antimicrobial activity in fresh chicken sausage. Ciência Rural 2018;48(11):e20180477. doi:10.1590/0103-8478cr20180477.
[19] Boriy GE, Yousef EA, Abd Rabou EA. Preparation and evaluation of pan bread from chia seeds powder as substitute wheat flour. Asian Food Sci J 2021;20(5):1–10. doi:10.9734/afsj/2021/v20i530294.
[20] Salehi F. Recent applications of powdered fruits and vegetables as novel ingredients in biscuits: A review. Nutrire 2020;45:1–10. doi:10.1186/s41110-019-0103-8.
[21] Hussain A, Kausar T, Sarwar A, Sarwar S, Kauser S, Chaudhry F, *et al*. Evaluation of total antioxidant and oxidant status, oxidative stress index and dpph free radical scavenging activities of pumpkin (cucurbita maxima) seeds ethanolic extracts. Turkish J Agr-Food Sci Tech 2022;10(sp2):2946–2950. doi:10.24925/turjaf.v10isp2.2946-2950.5630.
[22] Abd-El Fattah SM, Yahia Hassan A, Bayoum HM, Eissa HA. The use of lemongrass extracts as antimicrobial and food additive potential in yoghurt. J American Sci 2010;6:582–594.
[23] Kukade DS, Talib MI, Parate VR, Shrirame V. Development of lemongrass flavored high nutrient cookies by fortification of flaxseed and poppy seed flour. Int J Eng Res Technol 2015;4(10):221–225. doi:10.17577/IJERTV4IS100248.
[24] Thorat PP, Sawate AR, Patil BM, Kshirsagar RB. Effect of lemongrass powder on proximate and phytochemical content of herbal cookies. J Pharm Phytochemistry 2017;6(6):155–159.
[25] Hussain A, Kausar T, Majeed MA, Aslam J, Imtiaz M, Haroon H, *et al*. Development of nutritional biscuits for children, rich in Fe and Zn, by incorporation of pumpkin (Cucurbita maxima) seeds powder; a healthy pharma food in current post COVID 19 period. Pure Appl Biol 2022;12(1):392–403. doi:10.19045/bspab.2023.120042.
[26] American Association of Cereal Chemists. Aproved Methods of the American Association of Cereal Chemists. 10th ed. Minnesota: Inc. St. Paul; 2000.
[27] Alemayehu D, Desse G, Abegaz K, Desalegn BB, Getahun D. Proximate, mineral composition and sensory acceptability of home-made noodles from stinging nettle (Urtica simensis) leaves and wheat flour blends. Int J Food Sci Nutr Eng 2016;6(3):55–61. doi:10.5923/j.food.20160603.02.
[28] Shen Y, Jin L, Xiao P, Lu Y, Bao J. Total phenolics, flavonoids, antioxidant capacity in rice grain and their relations to grain color, size and weight. J Cereal Sci 2009;49(1):106–111. doi:10.1016/j.jcs.2008.07.010.
[29] Bao J, Cai Y, Sun M, Wang G, Corke H. Anthocyanins, flavonols, and free radical scavenging activity of Chinese bayberry (Myrica rubra) extracts and their color properties and stability. J Agric Food Chem 2005;53(6):2327–2332. doi:10.1021/jf048312z, PMID:15769176.
[30] de Carvalho LMJ, Gomes PB, de Oliveira Godoy RL, Pacheco S, do Monte PHF, de Carvalho JLV, *et al*. Total carotenoid content, α-carotene and β-carotene, of landrace pumpkins (Cucurbita moschata Duch): A preliminary study. Food Res Int 2012;47(2):337–340. doi:10.1016/j.foodres.2011.07.040.
[31] Brand-Williams W, Cuvelier ME, Berset CLWT. Use of a free radical method to evaluate antioxidant activity. LWT-Food Sci Technol 1995;28(1):25–30. doi:10.1016/S0023-6438(95)80008-5.
[32] Tsikritzi R, Moynihan PJ, Gosney MA, Allen VJ, Methven L. The effect of macro- and micro-nutrient fortification of biscuits on their sensory properties and on hedonic liking of older people. J Sci Food Agric 2014;94(10):2040–2048. doi:10.1002/jsfa.6522, PMID:24318046.
[33] Steel R, Torrie J, Dickey D (eds). Principles and procedures of statistics A biometrical approach. 3rd ed. New York: McGraw Hill Book Company Inc; 1997:334–381.
[34] Radwan H, Elmaadawy A. Potential Effects of Toast Bread Supplemented with Lemon Grass Powder on Kidney and Liver Functions in Rats. J Home Econ-Menofia University 2022;32(1):151–162.
[35] Asaolu MF, Oyeyemi OA, Olanlokun JO. Chemical compositions, phytochemical constituents and in vitro biological activity of various extracts of Cymbopogon citratus. Pak J Nutr 2009;8(12):1920–1922. doi:10.4314/sinet.v44i2.7.
[36] Birhanu N, Yohannes W, Chandravanshi BS. Determination of selected major and trace metals in lemongrass (Cymbopogon citratus) by microwave plasma-atomic emission spectrometry. Ethiopian J Sci 2021;44(2):205–214. doi:10.4314/sinet.v44i2.7.
[37] Ranade SS, Thiagarajan P. Lemon grass. Int J Pharm Sci Rev Res 2015;35(2):162–167.
[38] Algarni EHA. Nutritive Value of Sponge Cake and Pancakes Fortified with Bioactive Compounds and Antioxidant of Pumpkin Flour. J Biochem Technol 2020;11(2):24–32.
[39] Falah S, Ayunda RD, Faridah DN. Potential of lemongrass leaves extract (Cymbopogon citratus) as prevention for oil oxidation. J Chem Pharm Res 2015;7(10):55–60.
[40] Nguyen C, Mehaidli A, Baskaran K, Grewal S, Pupulin A, Ruvinov I, *et al*. Dandelion Root and Lemongrass Extracts Induce Apoptosis, Enhance Chemotherapeutic Efficacy, and Reduce Tumour Xenograft Growth In Vivo in Prostate Cancer. Evid Based Complement Alternat Med 2019;2019:2951428. doi:10.1155/2019/2951428, PMID:31391857.
[41] Mohamed SS, El-Sedeek LE, Seif MM, Amer MM, Mohamed SR, Abdel-Aziz MHM. Protective effect of Cymbopogon schoenanthus extract against formalin hazard in rats. Res J Med Plant 2017;11(1):8–13. doi:10.3923/rjmp.2017.8.13.
[42] Akoja SS, Coker OJ. Physicochemical, functional, pasting and sensory properties of wheat flour biscuit incorporated with Okra powder. Int J Food Sci Nutr 2018;3(5):64–70.
[43] Hussain A, Kauser T, Aslam J, Quddoos MY, Ali A, Kauser S, *et al*. Comparison of different techno-functional properties of raw lemon pomace and lemon pomace powder, and development of nutritional biscuits by incorporation of lemon pomace powder. Caraka Tani: J Sustain Agr 2023;38(1):176–192. doi:10.20961/carakatani.v38i1.67769.
[44] Yadav KC, Bhattarai S, Shiwakoti LD, Paudel S, Subedi M, Pant BR, *et al*. Sensorial and chemical analysis of biscuits prepared by incorporating Moringa flower powder and leaf powder. Int J Food Prop 2022;25(1):894–906. doi:10.1080/10942912.2022.2069807.
[45] Dias NAIS, Bandara GMWR. Nutritive assessment of composite flour biscuit incorporated with herbal plant powder. J Food Agr 2019;12(1):17–28. doi:10.4038/jfa.v12i1.5217.
[46] Gupta UC, Gupta SC. Sources and deficiency diseases of mineral nutrients in human health and nutrition: a review. Pedosphere 2014;24(1):13–38. doi:10.1016/S1002-0160(13)60077-6.
[47] Da Silva DJC. Nutritional and health benefits of carrots and their seed extracts. Food Nutr Sci 2014;5(22):2147. doi:10.4236/fns.2014.522227.
[48] Bolarinwa IF, Aruna TE, Raji AO. Nutritive value and acceptability of bread fortified with moringa seed powder. J Saudi Society Agr Sci 2019;18(2):195–200. doi:10.1016/j.jssas.2017.05.002.
[49] Agrahar-Murugkar D. Food to food fortification of breads and biscuits with herbs, spices, millets and oilseeds on bio-accessibility of calcium, iron and zinc and impact of proteins, fat and phenolics. LWT 2020;130:109703. doi:10.1016/j.lwt.2020.109703.
[50] Hassan HM, Aboel-Ainin MA, Ali SK, Darwish AGG. Antioxidant and Antimicrobial activities of MEOH Extract of Lemongrass (Cymbopogon citratus. J Agr Chem Biotechnol 2021;12(2):25–28. doi:10.21608/jacb.2021.149473.
[51] Kusmardiyani S, Alfianti F, Fidrianny I. Antioxidant profile and phytochemical content of three kinds of lemongrass grown in West Java-Indonesia. Asian J Pharm Clinic Res 2016;9(4):381–385.
[52] Aboagye G, Tuah B, Bansah E, Tettey C, Hunkpe G. Comparative evaluation of antioxidant properties of lemongrass and other tea brands. Sci African 2021;11:e00718. doi:10.1016/j.sciaf.2021.e00718.
[53] Gazwi HSS. Preventive effect of lemongrass (Cymbopogon citratus) against oxidation in soybean oil. Proceedings of the National Academy of Sciences, India Section B: Biological Sciences 2020;90:151–159. doi:10.1007/s40011-019-01091-z.
[54] Shafi M, Baba WN, Masoodi FA, Bazaz R. Wheat-water chestnut flour blends: effect of baking on antioxidant properties of cookies. J Food Sci Technol 2016;53(12):4278–4288. doi:10.1007/s13197-016-2423-5, PMID:28115768.
[55] Valenzuela GM, Soro AS, Tauguinas AL, Gruszycki MR, Cravzov AL, Giménez MC, *et al*. Evaluation polyphenol content and anti-

oxidant activity in extracts of *Cucurbita* spp. OALib 2014;1(3):1–6. doi:10.4236/oalib.1100414.

[56] Akgul H, Mohammed FS, Kına E, Uysal İ, Sevindik M, Doğan M. Total Antioxidant and Oxidant Status and DPPH Free radical activity of Euphorbia eriophora. Turk J Agr-Food Sci Technol 2022;10(2):272–275. doi:10.24925/turjaf.v10i2.272-275.4685.

[57] Ismail T, Akhtar S, Riaz M, Ismail A. Effect of pomegranate peel supplementation on nutritional, organoleptic and stability properties of cookies. Int J Food Sci Nutr 2014;65(6):661–666. doi:10.3109/09637486.2014.908170, PMID:24725173.

[58] Hussain A, Kausar T, Sehar S, Sarwar A, Ashraf AH, Jamil MA, *et al*. Determination of total phenolics, flavonoids, carotenoids, β-carotene and DPPH free radical scavenging activity of biscuits developed with different replacement levels of pumpkin (Cucurbita maxima) peel, flesh and seeds powders. Turk J Agr-Food Sci Technol 2022;10(8):1506–1514. doi:10.24925/turjaf.v10i8.1506-1514.5129.

[59] Choi HY. Antioxidant activity and quality characteristics of pine needle cookies. J Korean Society Food Sci Nutr 2009;38(10):1414–1421. doi:10.3746/jkfn.2009.38.10.1414.

[60] Kieling DD, Prudencio SH. Antioxidants properties, physicochemical, and sensory quality attributes of a new soy-based beverage with lemongrass and lime juice. J Culinary Sci Technol 2019;17(2):103–117. doi:10.1080/15428052.2017.1405859.

[61] Abdel-Naeem HHS, Elshebrawy HA, Imre K, Morar A, Herman V, Pașcalău R, *et al*. Antioxidant and Antibacterial Effect of Fruit Peel Powders in Chicken Patties. Foods 2022;11(3):301. doi:10.3390/foods11030301, PMID:35159453.