International Journal of Chemical Studies

P-ISSN: 2349–8528
E-ISSN: 2321–4902
IJCS 2017; 5(6): 758-762

Received: 14-09-2017
Accepted: 17-10-2017

Thorat PP
Department of Food Engineering
College of Food Technology,
VNMKV, Parbhani,
Maharashtra, India

Sawate AR
Department of Food Engineering
College of Food Technology,
VNMKV, Parbhani,
Maharashtra, India

Patil Bm
Department of Food Engineering
College of Food Technology,
VNMKV, Parbhani,
Maharashtra, India

Kshirsagar RB
Department of Food Engineering
College of Food Technology,
VNMKV, Parbhani,
Maharashtra, India

Correspondence
Thorat PP
Department of Food Engineering
College of Food Technology,
VNMKV, Parbhani,
Maharashtra, India

Proximate and phytonutrient content of *Cymbopogon citratus* (Lemongrass) leaf extract and preparation of herbal cookies

Thorat PP, Sawate AR, Patil Bm and Kshirsagar RB

Abstract
Cymbopogon citratus is a perennial grass belonging to family *Graminaceae* and grouped under genus *Cymbopogon*. In this study, the herbal cookies were prepared by incorporating *Cymbopogon citratus* extract at 0, 1, 3 and 5% level. The proximate and phytochemical analysis of fresh lemongrass leaves and its aqueous extract were analyzed. The phytochemical constituents were found to be less as compared to fresh leaves. The moisture, protein, lipid, crude fiber and ash content were increased while lipid content were decreased after incorporation of extract in cookies. The herbal cookies were found to increase in proximate composition with increase in level of lemongrass powder. The herbal cookies contain alkaloid (0.14%), saponin (0.10%), tannin (0.054%), steroids (0.048%), phenols (0.174%) and flavonoids (0.18%) in sensorially selected sample. The sample with 3% lemongrass powder was found to be highly acceptable over other sample. The required daily dose of was about 0.7 mg/kg of bodyweight/day.

Keywords: Herb, hcookies, *Cymbopogon citratus*, leaf extract, lemongrass

Introduction
The word herb is derived from the Latin 'herba,' meaning grass or, by extension, green crop. Herb was originally applied to a wide range of leafy vegetables. Herbs are seed plants that do not produce woody stems like a tree and will live long enough to develop and produce flowers and seeds. India is the largest producer of medicinal herbs and is called as botanical garden of the world (Grover *et al.*, 2002) [1]. In the last few years there has been an exponential growth in the field of herbal medicine and functional foods. The population everywhere desires to eat a healthier diet without changing their conventional dietary patterns (Becker and Kyle, 1998) [2].
The lemongrass (*Cymbopogon citratus* (Steud) Wats) is a perennial grass belonging to family *Graminaceae* and grouped under genus *Cymbopogon*. The leaf height is about 100 cm in length and 2 cm in width. When squeezed, the leaves usually produce yellow or amber coloured, aromatic essential oil (Adejuwon and Esther, 2007) [3]. Its aqueous extract is commonly used as an aromatic drink while the whole plant is well incorporated into traditional food for its lemon flavour. It also enjoyed wide application in folk medicine (Figueirinha *et al.*, 2008) [4].
Aqueous extracts of dried leaves are used in traditional medicine for the treatment of inflammation, digestive disorders, diabetes, nervous disorders, and fever, as well as other health problems (Carbajal *et al*., 1989) [5]. However, the mechanism of action of Cy is poorly explored and characterized, namely the mechanism responsible for its anti-inflammatory effects. In addition, this extract and its polyphenolic fractions inhibit LPS-induced NO production and iNOS expression in fetal skin-derived dendritic cell line (FSDC) (Figueirinha *et al*., 2010) [4], reinforcing the potential use of *cymbopogon* extract as source of a new anti-inflammatory drug.
Cookies can be classified as ready to eat and convenient foods. Traditionally, the process of cookies making are fairly simple with basic ingredients consist of flour, eggs and sugar. Generally, cookies are recognized as flat, hard and crunchy food. Normally, cookies are classified according to their method of preparation such as drop, moulded, presses, refrigerated, bar or rolled. Apart from that, the dominant ingredients that been used in the formulation also commonly being used to classify the cookies, for example, nut cookies, fruit cookies, herbal cookies and chocolate cookies (Norhidayah *et al*., 2014) [6].

In the present study, herbal cookies are formulated based on optimal level of incorporating all selected ingredients for the acceptable level and it was evaluated based on sensory properties as response variables. The level of *cymbopogon citratus* extract in preparation of cookies was optimized and its effect on proximate and phytonutrient content were analysed.

Materials and Methods

The *Cymbopogon citratus* was collected from the Department of Botany, College of Agriculture, Vasantrao Naik Marathwada Krishi Vidyapeeth, Parbhani. The research was carried out in College of Food Technoogy, VNMKV, Parbhani.

Preparation of *Cymbopogon citratus* leaf extract

Fresh, young leaves of lemon grass were collected, authenticated, washed and soaked in water for 3 hours. The leaves were then allowed to dry at room temperature under a clean cloth for 24 hours. The dried leaves were then ground to fine powder. 30 g of the dry powder was weighed and was used for extract preparation.

Extracts for the plant leaves were prepared using distilled water (aqueous extract) as solvents. Extracts were prepared using the soxhlet apparatus. The extracts were diluted appropriately before use (Harborne, 1987) [7].

Preparation of herbal cookies

Firstly, oven was preheated at 170°C. The butter was beaten in a food mixer and sugar was added. Then, sifted flour sample was added in the mixture. The mixture was then mixed and kneaded until it became dough. The dough was moulded into balls, placed on the greased tray and finally, baked in the oven for 15 minutes until a golden brown colour was obtained. They were allowed to cool before being packed in polyethylene bag and stored in cold and dry place until further analysis being done (Norhidayah *et al*., 2014) [6].

Table 1: Standard Recipe for Cookies

Ingredients	Amount (g)
Maida	100
Sugar	60
Fat	45
Baking Powder	1.5
Ammonium Bicarbonates	1.5
Milk	As Required

Table 2: Formulation of Composite flour (%) for Cookies

Sample	Maida (%)	*C. citratus* extract (%)
Control	100	0
A	99	1
B	97	3
C	95	5

Proximate analysis

The proximate composition like moisture, protein, lipid, crude fibre and ash were measured as per standard method given in AOAC, (1990) [8].

Phytochemical Analysis

Determination of alkaloid content

Five gram of the sample was weighed into a 250 ml beaker and 200 ml of 10% acetic acid in ethanol was added and allowed to stand for 4 min, this was filtered and extract was concentrated on a water bath to one quarter of the original volume. Concentrated ammonium hydroxide added drop wise to the extract until the precipitation was completed. The whole solution was allowed to settle and the precipitate was collected and washed with dilute ammonium hydroxide and then filtered. The residue was alkaloid which was dried and weighed (Harbone, 1973) [7].

$$\text{Alkaloid (\%)} = \frac{W_3\text{-}W_2}{W_1} \text{X100}$$

Where, W_1 =initial weight of sample,
W_2 =weight of the extract and
W_3 = final weight of the residue

Determination of flavonoid content

Ten gram of the sample was extracted repeatedly with 100 ml of 80% aqueous methanol at room temperature. The whole solution was filtered using Whatman filter paper No. 42 (125 mm). The filtrate was transferred into crucible and evaporated into dryness over water bath and weighed to a constant weight (Bohm, 1994) [9].

Determination of tannin content

Finely grounded sample was weighed (0.2g) into a 50 ml sample bottle. Ten of 70% aqueous acetone was added and properly covered. The bottle was put in an ice bath shaker and shaken for 2 hrs at 300ºC. The solution was then centrifuge and the supernatant stored in ice, 0.2 ml of the solution was pipette into the test tube and 0.8 ml of distilled water was added. Standard tannin acid solution was prepared from a 0.5 mg/ml of the stock and the solution made up to 1ml with distilled water, 0.5 ml of Folinciocateau reagent was added to the sample and standard followed by 2.5 ml of 20% Na_2CO_3 the solution was then vortexed and allowed to incubate for 40 min at room temperature, its absorbance was read at 725 nm against a reagent blank concentration of the same solution from a standard tannic acid curve prepared (Markkar, 1996) [10].

Determination of saponin content

Two gram of the finely grinded sample was weighed into a 250 ml beaker and 100 ml of Isobutyl alcohol was added. Shaker was used to shake the mixture for 5h to ensure uniform mixing. The mixture was filtered using No 1 Whatman filter paper into 100 ml beaker containing 20 ml of 40% saturated solution of magnesium carbonate. The mixture obtained again was filtered using Whatman filter paper No 1 to obtain a clean colourless solution. One (1 ml) was added into 50 ml volumetric flask using pipette, 2 ml of 5% iron (iii) chloride ($FeCl_3$) solution was added and made up to the mark with distilled water. It was allowed to stand for 30 min for the colour to develop. The absorbance was read against the blank at 380 nm (Bruneton, 1999) [11].

$$\text{Saponin} = \left[\frac{\text{Absorbance of sample x concentration of standard}}{\text{Absorbance of standard}}\right] \text{-1}$$

Determination of total phenol content

Preparation of acetone extract

The sample was homogenized with chilled phosphate buffer (0.05M, p^H 7.5) using a homogenizer. The homogenate was made up to 80% acetone with respect to acetone by adding chilled acetone and mixed thoroughly and filtered using cheesecloth. The residue was washed with 80% chilled

acetone, filtered and air dried (acetone powder). The filtrates (80% acetone extract) were combined and kept in 4^0C for further studies. The extractability of 80% ethyl alcohol forth extraction of bioactive compounds from orange and pomace peel also carried out in the same way using ethyl alcohol instead of acetone.

The 80% acetone extract used for the estimation of total phenolic compounds, anthocyanin and carotenoid contents and evaluated for antioxidant activity. The dried powder obtained after filtering was used for the estimation of dietary fibre and enzymes

Determination of total phenolic content

The sample extracts with 80% acetone, 80% ethanol and 0.05M sodium phosphate buffer (pH 7.5) were centrifuged for 15 min at 10,000 X g. The clear supernatants obtained were subjected to total polyphenol estimation using the method of Swain and Hills (1959) [12]. The 0.5 ml of extract, 4.5 ml of ethanol was added and to this 0.5 ml phenol reagent (Folin-Ciocalteu reagent, diluted 1:2 with water) was added and the contents were incubated at room temperature for 3 min to this 1 ml of saturated Na_2Co_3 was added and the reaction mixture was incubated at room temperature for 60 min. The absorbance was recorded at 675 nm; Gallic acid was used as a standard. The total polyphenol content in the extract was expressed as gallic acid equivalents (GAE).

Sensory Analysis

The prepared herbal cookies were organoleptically evaluated on the basis of 9-point hedonic scale. The level of addition of *C. citratus* extract in cookies were optimized on the basis of sensory score.

Results and Discussion

Proximate Composition of *Cymbopogon citratus* leaves

The proximate composition of lemongrass leaves was carried out and results obtained were tabulated in Table 3.

Table 3: Proximate composition of lemongrass leaves

Content	Value
Moisture (%)	71.03
Protein (%)	3.83
Carbohydrate (%)	20.73
Fat (%)	4.76
Fibre (%)	9.30
Ash (%)	2.94

The fresh green leaves of lemongrass plant were collected and proximate analysis was determined. The moisture content (71.03%) of lemongrass is desirable as it will prevent microbial attack and allow for high storage capacity due to its antioxidant activity. The protein and carbohydrate content of leaves were found to be 3.83 and 20.73%. This shows that *cymbopogon citratus* is a good source of energy. The fat, fibre and ash content of lemongrass leaves were 4.76, 9.30 and 2.94% respectively. These findings are in correlation with the results obtained by Assous *et al*. (2013) [13].

Phytochemical constituents of lemongrass (*Cymbopogon citratus*) leaves

The values of phytochemicals were presented in Table 4.

Table 4: Phytochemical constituents of lemongrass leaves

Constituents	Values
Alkaloid (g/100g)	1.27
Saponin (g/100g)	1.06
Tannin (g/100g)	0.87
Phenols (g/100g)	0.27
Flavonoids (g/100g)	0.84

The data in Table 4, represent that the alkaloid and saponin content in lemongrass leaves were 1.27 and 1.06%. The tannin content (0.87%) which attributed to astringency in prepared product. The antioxidant activity of leaves was dependent on phenol and flavonoid content which was found to be 0,27 and 0.84% in *cymbopogon citratus* leaves. The similar results were also obtained by Ndukwe *et al*. (2013) [14].

Proximate composition of *c. citratus* extract

The proximate composition of *c. citratus* extract were analysed and results presented in Table 5.

Table 5: Proximate composition of *cymbopogon citratus* extract

Parameters	Value (%)
Moisture	11.15
Protein	0.30
Lipid	1.05
Crude fibre	81.11
Ash	6.20

The results presented in Table 5 revealed that, the *c. citratus* had less moisture content (11.15%). The protein, lipid and ash content of *c. citrtus* was found to be 0.30, 1.05 and 6.20% respectively. The high amount of crude fibre were found in leaf extract of *c. citratus i.e.* 81.11%. The findings in the study are similar with the results obtained by Akande *et al*., (2012) [15].

Phytochemical constituents of *c. citratus* extract

The phytochemical constituents represent the medicinal value of plant as they help in treatment of different types of diseases. The data for phytochemical constituents of *c. citratus* leaf extract is presented in Table 6.

Table 6: Phytochemical constituents of *c. citratus* extract

Constituents	Content (g/100g)
Alkaloids	0.47
Saponin	0.36
Tannin	0.14
Steroid	0.1
Phenol	0.31
Flavonoid	0.35

The Table 6 showed, the values for alkaloid, saponin and tannin content of *c. citratus* extract were 0.47, 0.36 and 0.14% respectively. Alkaloids are formed as metabolic by-product and have been reported to be responsible for the antibacterial activity. Pure isolated plant alkaloids and their synthetic

derivatives are used as basic medicinal agent for analgesic, anti pasmiodic and bacterial effects (Okwu, 2004) [16]. Saponins are glycosides which include steroid and triterpenoid saponins. It has hypocholesterolmic effects and thus may aid in lessening the metabolic burden on liver. Plant saponin help human to fight fungal infection, combat microbes and viruses, and knock out some kind of tumor cells particularly lung and blood cancer. They also lower blood cholesterol thereby reducing the heart disease. (Olivebever, 1986) [17].

The steroid, phenol and flavonoid content in *c. citratus* were found to be 0.1, 0.31 and 0.35% respectively. Flavonoids are phenolic compounds that serve as flavouring ingredients of plant leaves. As antioxidant flavonoid helps in digestion and assimilation of food to the body system and treatment of capillary and vascular weakness, varicose veins, blood clotting and dysfunction (Roger, 2002) [18]. Tannins are plant polyphenols which have medicinal properties such as antimicrobial, anti-inflammatory and astringent activity (Chung *et al*., 1998) [19]. The results obtained for phytoconstituents in *c. citratus* are in agreement with the report of Ndukwe *et al*. (2013) [14].

Proximate composition of *c. citratus* added herbal cookies

The proximate composition of herbal cookies was analysed at various level of *c. citratus* extract (1, 3 and 5%). The values were tabulated in Table 7.

Table 7: Proximate composition of *c. citratus* added herbal cookies

Treatment	Moisture (%)	Ash (%)	Lipid (%)	Protein (%)	Crude fiber (%)
T_0	4.43	2.54	24.01	6.68	0.22
T_1	4.48	2.61	20.50	8.64	2.47
T_2	4.52	3.55	16.75	10.10	4.15
T_3	4.55	5.63	13.44	11.28	6.34

T_0 – Cookies without *c. citratus* extract
T_1 – Cookies with 1% *c. citratus* extract
T_2 – Cookies with 3% *c. citratus* extract
T_3 – Cookies with 5% *c. citratus* extract

From the above Table, it could be concluded that there was increase in moisture, proteins, ash and crude fibre whereas lipid content were reduced (Table 7). The moisture content was increased from 4.43% to 4.55% due to high moisture content of extract as compared to raw cookies. The protein (6.68 to 11.28%), crude fibre (0.22 to 6.34%) and ash (2.54 to 5.63%) were increased while lipid (24.01 to 13.44%) was reduced due to the content of *c. citratus* extract. These results were found to be similar with Dhillon *et al*., (2013) [20] who found that the fibre content were increased with addition of oregano herb.

Phytochemical constituents of *c. citratus* added herbal cookies

The prepared herbal cookies with incorporation of *c. citratus* extract were subjected to phytonutrient analyses and results are tabulated in Table 8.

Table 8: Phytochemical constituents of *c. citratus* added herbal cookies

Constituents	Content		
	1%	3%	5%
Alkaloids (g/100g)	0.04	0.14	0.24
Saponin (g/100g)	0.036	0.108	0.18
Tannin (g/100g)	0.018	0.054	0.09
Steroid (g/100g)	0.016	0.048	0.08
Phenol (g/100g)	0.058	0.174	0.29
Flavonoid (g/100g)	0.06	0.18	0.30

It could be concluded from Table 8 that; the phytonutrient contents were increased as increase in extract level. The results found that alkaloid, saponin, tannin, steroid, phenol and flavonoid content of herbal cookies with 5% extract were 0.24, 0.18, 0.09, 0.08, 0.29 and 0.30 respectively.

The baking did not affect the levels of TPC or FRAP AA relative to the flour and dough, but slightly decreased the levels of TFC. This might be due to omission of a pre-soaking stage before baking, and thus no discarding of the baking water. These results strengthen our previous observation that poly- phenols and flavonoids most likely leak into the sur-rounding water during the soaking and cooking processes (Segev *et al*., 2011) [21]. In cv. Zehavit, however, a slight but significant in- crease in TPC and FRAP AA was observed in the bread crust. This might be due to the production of Maillard reaction products in the crust during thermal processing (Lindenmeier *et al*., 2004) [22].

Similar observations have been made when baking rhubarb, whereby both TPC and FRAP AA were higher during the first 20 min and then decreased to low levels (McDougall *et al*., 2010) [23], and when baking chocolate cookies and chocolate cakes made with baking powder rather than baking soda (Miller *et al*., 2006) [24].

Sensory analysis of Herbal cookies

The herbal cookies with incorporation of *c. citratus* extract were analysed for sensory parameters such as colour, flavour, texture, taste and overall acceptability on the basis of 9-point hedonic scale. It helps in deciding the level of incorporation of *c. citratus* extract as per sensory acceptance by consumer.

Table 9: Sensory analysis of Herbal Cookies

Samples	Colour	Flavour	Texture	Taste	Overall Acceptability
T_0	8.2	7.8	8.2	7.5	7.6
T_1	8.3	7.8	8.0	7.5	7.9
T_2	8.5	8.0	8.3	8.0	8.3
T_3	8.2	7.5	8.0	7.2	7.8

The Table 9 showed that the level of *c. citratus* extract effects on sensory parameters. The overall acceptability, texture and taste values are comparable with the control sample. The increased level of lemongrass powder than 3%, found that it adversely effects on sensory quality of prepared cookies.

Conclusion

The preparation of herbal cookies with incorporation of *c. citratus* leaves extract is better way to providing the nutritionally and medicinally rich bakery products. The prepared cookies were also found to contain phytochemical which plays the role such as anti-cancer, anti-diabetes, anti-inflammation agent in human body. As suggested safe limit for human (Based on experiments in rats) is 0.7/mg/kg of body weight/day (Fandohan *et al*., 2008) [25].

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