

Effect of Wheatgrass for Enhancing the Nutritional, Textural, Total Antioxidant and Sensory Characteristics of 'Idli'-An Indian Steamed Rice Cake

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Abstract: Wheatgrass is a rich source of nutrient and antioxidative compound. The incorporation of wheatgrass powder in to a traditional staple food and their effect on the textural and functional properties of the product have been studied. Freeze dried wheatgrass flower at levels of 0.05-3.0% was added to the formulation mix. The results obtained from the analysis of the fermented product are discussed in terms of the effect of wheatgrass powder addition on nutritional and textural characteristics. The samples were prepared using selected strain of microorganism *Leuconostoc mesenteroides* and *Lactobacillus plantarum*. Incorporation of wheatgrass in to the batter significantly ($p<0.05$) increased the level of phenolic compounds ($p<0.05$) but decreased the volume of the batter and volume of the product. Hardness of the product is indirectly proportional to the batter volume. Sensory test panel indicated that wheatgrass flower could be incorporated into the products up to the level of 1%.

Key words: Wheatgrass, antioxidant, HPLC, texture, sensory, flower

INTRODUCTION

Nowadays we find lots of fortified food in Indian food culture, like fortified salt, oil, cookies, margarine, etc. Paddy or rice is a major agricultural product in India. General food can gain their nutrition level if these are converted into fortified food. As observed, no attempt has been made to fortify idli, prepared from rice as food supplement. Cereal/legume-based foods are a major source of economical dietary energy and nutrients world-wide. Among the fermented food of India, idli, a fermented steamed product with a soft and spongy texture is a highly popular and widely consumed snack food in India. Traditionally, the product is made from naturally fermented batter prepared by mixing milled rice (*Oryza sativa*) and dehulled Blackgram dhal (*Phaseolus mungo*) in varying proportions (Desikachar *et al.*, 1960; Radhakrishnamurty *et al.*, 1961). The lactic acid bacteria *Leuconostoc mesenteroides*, *Streptococcus faecalis*, *Lactobacillus delbrueckii*, *Lactobacillus fermenti*, *Lactobacillus lactis* and *Pediococcus cerevisiae* have been found to be responsible for the fermentation process although, *L. mesenteroides* and *S. faecalis* are considered to be the microorganism essential for leavening of the batter and for acid production in idli (Ramakrishnan, 1993). Studies showed that water extracts of wheatgrass (*Triticum aestivum* L.) is a good source of

antioxidant. The antioxidant activity obtained from different assays show that a higher level of antioxidant is present in fresh wheatgrass of growth of 7-8 days. Extracts of home-grown wheatgrass under known environmental conditions can be used as a dietary supplement for antioxidant compounds such as polyphenols and flavonoids. Tender wheatgrass is a good source of essential trace elements (Kulkarni *et al.*, 2006).

Therefore, the principal objectives of this study were to develop a functional idli from wheatgrass flour blends and to evaluate effects of the addition of wheatgrass powder on the antioxidative activity, nutritional value, texture and general acceptability of the product.

MATERIALS AND METHODS

Raw materials: The major food ingredients used for the preparation of idli are Split Dehusked (SD) black gram dhal (*Phaseolus mungo* Roxb.) and parboiled rice (*Oryza sativa*) which was purchased from a local market. Seed of wheat (*Triticum aestivum*) and common salt, NaCl (Tata salt, India) were purchased from the local grocery stores of Kolkata.

Processing of fortifying ingredients: The seeds of wheat (*Triticum aestivum* L.) were collected from Kolkata,

West-Bengal were authenticated by the taxonomists of the Botanical Survey of India, Kolkata (Ref. CNH/1-1/10/2010/Tech II/176). Those seeds were washed with tap water and then with distilled water. The seeds were soaked in distilled water (24-25°C) for 5 h. The steeped seeds were then covered with a clean moistened cloth to promote the onset of germination for 24 h (120 mL of water was sprayed onto the cloth every 8 h to keep the cloth moistened). The seeds were then transferred to the tray. The wheat plants were grown in sterilized soil. The seeds were sowed in trays (length 35 cm×breadth 25 cm×depth 7 cm), containing 4 kg of soil (pH 7.5±0.4). The soil was provided with sufficient tap water regularly and was placed in a room where normal air flow and sunlight were available. Samples were collected from the plant on the 8th day. The samples collected were washed, wiped and cut into small pieces (2 cm length). Wheatgrass samples were frozen at -78°C (Ultra-Low Temperature Freezer, Model No.-C340-86, New Brunswick Scientific, England) for 12 h and then freeze-dried in a vacuum (2.4×10^{-2} mB) for 6 h. The condenser temperature was 49°C. The dried material had moisture content of 5.4% (AOAC, 1984). Then, dried wheatgrass was ground in kitchen mixer at 8000 rpm for 5 min to get a fine, smooth wheatgrass powder. This wheatgrass powder was used directly for fortification in idli.

Starter cultures and inoculum preparation: The cultures included were *Leuconostoc mesenteroides* (NCIM 2073) and *Lactobacillus plantarum* (NCIM 2084) which were available as stock cultures in the National Collection of Industrial Microorganisms (NCIM), National Chemical Laboratory, Pune, India. Strains of *L. mesenteroides* and *L. plantarum* were maintained at 4°C in Lactobacillus MRS Broth (Hi-Media Lab, Mumbai, India) and subcultured at 15 days interval. For use in the experiment, cultures were grown individually in 100 mL aliquots of Lactobacillus MRS Broth at 37°C for 48 h. Cells of the respective strains were harvested by centrifugation at 3000 rpm for 20 min at ambient temperature. The washed cells in sterile distilled water were suspended in sterile 0.85% NaCl solution. Appropriate dilutions of the cell suspensions were prepared in 0.85% NaCl solution to obtain initial counts of 2×10^2 cfu g⁻¹ of each *L. mesenteroides* and *L. plantarum* in the batter.

Idli batter preparation process: Idli were prepared with parboiled rice and split dehusked black gram dhal. The ideal ratio of rice and black gram dhal for the product is 2:1. The ingredients, rice and black gram dhal were washed several times with water to remove adhering dirt

and dust particles from the surface, soaked separately for 4 h at 30±1°C temperature and ground in a kitchen mixer blender separately. Rice was ground coarsely and black gram finely to a smooth batter and both were mixed together with common salt (2.0%). Two starter cultures in combination to get an initial level of 2×10^2 cfu g⁻¹ of each *L. mesenteroides* and *L. plantarum* was added to the prepared batter and mixed uniformly. The combined mixture was then allowed to ferment for 20 h at 30±1°C in glass beakers covered with cotton cloths. The mean particle size of parboiled rice and decorticated black gram batter prepared was 400-250 µm measured by 40-60 mesh size sieves. Test samples include preparation of white control idli and idli fortified with 0.5, 1.0, 2.0 and 3.0% (w/w) of the wheatgrass powder. The control idli is designated as 'CONT' and the fortified idli are designated as 'P' (Idli with 0.5% wheatgrass powder), 'Q' (Idli with 1% wheatgrass powder), 'R' (Idli with 2% wheatgrass powder) and 'S' (Idli with 3% wheatgrass powder). The final product is obtained by cooking the fermented batter in steam for 20 min.

Evaluation of idli batter

Evaluation of increase in volume: For evaluation of percent increase in volume, the batter was placed in a measuring cylinder (Nisha *et al.*, 2005).

pH measurement: The pH of the batter at different fermentation times was recorded using a pH meter (Thermo Orion Basic pH Meter, Model 420A pH/mV/ORP/temperature meter).

Total lactic acid concentration: The total lactic acid concentration was determined by the technique described by Balaubramanian and Viswanathan (2007).

Vitamin estimation by HPLC: Liquid chromatography was performed with a HPLC (Jasco, LC-Net II/ADC, Japan). Methanol (HPLC grade) and K₂HPO₄ (extra pure) were procured from Merck. Water used in all the experiments was doubly distilled and deionised (Membrapure Aquinity P3/P7, Germany). The vitamin standards (ascorbic acid, niacin, pantothenic acid, pyridoxine, thiamine and folic acid) were of analytical-reagent grade procured from Sigma (India), Merck (India), Loba-Chemie (India) and were used as such without purification. Stock and standard solutions of water-soluble vitamins were prepared in mobile phase.

Idli consists of many components that cause chromatographic interferences with vitamins. For this reason, the sample treatment proposed consisted of Solid

Phase Extraction (SPE) with Sep-Pak C₁₈ cartridges (Samplic, 500 mg, Agilent Technologies, USA) that enable separation of water-soluble vitamins and remove most of the interfering components. Four parts of deionised water (20 g) were added into one part of idli (5 g) (dilution factor, F = 5). The mixture was homogenized using a homogenizer at medium speed for 1 min. The homogenized samples were centrifuged for 10 min at 14×10^3 g at 4°C (Hanil, Supra 22K, Korea). The SPE Method of Cho *et al.* (2000) was used for the extraction of water-soluble vitamins. The stationary phase was flushed with 10 mL methanol and 10 mL water was adjusted to pH 4.2 to activate the stationary phase. Homogenized and centrifuged idli (10 mL) was then loaded. Acidified water was prepared by adding a 0.005 M HCl solution drop by drop with stirring until the pH reached a predetermined value. The sample was eluted with 5 mL water (pH 4.2) then 10 mL methanol at a flow rate of 1 mL min⁻¹. The eluent was collected in a bottle and evaporated to dryness. The residue was dissolved in mobile phase. Before HPLC analysis, all samples were filtered through 0.45 µm pore size filters (Millipore). Samples (20 µL) of solutions of the water-soluble vitamins were injected into the HPLC column.

The column eluate was monitored with a photodiode-array detector at 210 nm for all the vitamins. The mobile phase was filtered through a 0.45 µm membrane and degassed by sonication before use. The mobile phase was 0.1 mol L⁻¹ KH₂PO₄ (pH 7)-methanol, 90:10. The flow-rate was 0.7 mL min⁻¹. The C₁₈ column was operated at room temperature (25°C). Chromatographic peak data were integrated up to 15 min. Identification of compounds was achieved by comparing their retention times and UV spectra with those of standards stored in a data bank. Concentrations of the water-soluble vitamins were calculated from integrated areas of the sample and the corresponding standards.

Evaluation of idli

Bulk density: Bulk density (g/c.c) was measured by seed displacement method using mustard seeds (g/c.c) (Nisha *et al.*, 2005).

Textural profile analysis: Idli has a circular shape of approximately 5-10 cm diameter (depending on the mold size), flat with lower and upper surface bulging so that the product is thick at the center (2-3 cm) and tapering towards periphery. Texture of the idlis was analyzed with Instron Universal Testing Machine, Table Model 4301 (Instron Ltd. High Wycombe, Bucks, UK) in the compression mode fitted with a 50 N load cell. All test

samples are 20 mm thick. Double compression test was performed by compressing axially each sample with a 40 mm diameter flat plate probe attached to the moving crosshead. The testing conditions were: compression ratio of 50% deformation from the initial height of the sample; 10 mm min⁻¹ crosshead speed 10 mm min⁻¹ chart speed. The force-distance curve obtained was used to derive the various textural properties (Bourne, 1978).

Color: Color of the idlis was measured using Hunter Lab Colorimeter Model DP-9000 D25A (Hunter associates laboratory, Reston, VA, USA), in terms of Hunter parameters L (lightness, ranging 0-100 indicating black to white), a (+a; redness and -a; greenness) and b (+b; yellowness and -b; blueness). Five replicates of color measurements were taken. The combination parameters were calculated by using tristimulus values measured. Hue angle values were calculated by using equation, Hue angle = $\tan^{-1} |b/a|$ (Baysal *et al.*, 2003; Heimdal *et al.*, 1995).

Antioxidant analysis

Total phenols and total flavonoids: For determining both total phenolic and flavonoid contents, calibration curves were obtained using known quantities of standard antioxidants. For preparing the extract, idli samples were sliced (2 cm width and 2 cm thickness) and dried in an oven at 30°C for 12 h. The dried material was ground in a grinder to obtain powdered idli. The powdered product was then extracted with 80% aqueous methanol (1 g/10 mL) for 2 h at 37°C.

Samples were then centrifuged at 10,000×g for 15 min. The supernatant collected was used in the antioxidant assay. The total phenolic content of ethanol extracts were measured using a modified Folin-Ciocalteu Method (Lowry *et al.*, 1951). The absorbance was measured at 750 nm. The measured values were compared with a standard curve of gallic acid concentrations and expressed as millimoles of gallic acid equivalents/100 g fresh material. The flavonoid contents of the extracts were also measured (Luximon-Ramma *et al.*, 2002). The absorbance was measured at 368 nm. The values obtained were compared with a standard curve of catechin concentrations and expressed as millimoles of catechin equivalents/100 g fresh material.

DPPH radical scavenging activity: The antioxidant activity of the extracts on the basis of the scavenging activity of the stable 1,1-Diphenyl-2-Picrylhydrazyl (DPPH) free radical was determined by the method described by Sreelatha and Padma (2009).

Aqueous methanolic extract (0.1 mL) was added to 3 mL of a 0.004% MeOH solution of DPPH. Absorbance at 517 nm was determined after 30 min and the percent inhibition activity was calculated as $((A_o - A_e)/A_o) \times 100$ (A_o = Absorbance without extract; A_e = absorbance with extract).

Sensory analysis: Idli samples were coded and presented to 30 panel members for sensory scoring. The panel members who were familiar with sensory analysis techniques were postgraduate students and research scholars of the Department of Food Technology and Bio-chemical Engineering (Jadavpur University, India). Three sets of blend ratio samples were analyzed on separate occasions. Water was used for mouth rinsing before and after each sample testing. Each set contained one control (without any fortifying ingredients) and four samples prepared with different fortifying ingredients. Samples were scored for appearance, taste, colour, texture, aroma and overall acceptability according to numerical scoring system. The model used in this analysis was an acceptance test on the hedonic scale with values ranging from “1” (extremely disliked) to “9” (extremely liked). The sensory analysis data were subjected to statistical analysis. Mean and standard deviation were individually calculated for scores obtained for all quality attributes of each product.

Statistical analysis: All the studies were replicated three times and the mean and standard deviation were individually calculated for scores obtained for all quality attributes of each product. All the experimental data were analyzed statistically for Analysis of Variance (ANOVA) in the Microsoft Excel 2007. Means were compared by Fisher's least significant difference test at a significance level of $p \leq 0.05$ (Gacula and Singh, 1984).

RESULTS AND DISCUSSION

Evaluation of percentage variation of wheatgrass on quality parameters of herbal idli batter

Analysis of batter volume: As compared to control batter with no fortifying ingredients, the batter with fortifying ingredients showed lesser increase in volume (Fig. 1). The volume of the batter was observed to decrease with the increase in the substitution level with wheatgrass powder. The highest reduction is in the batter made from wheatgrass powder at 3% level of substitution. The control sample shows a volume increase of 56% whereas

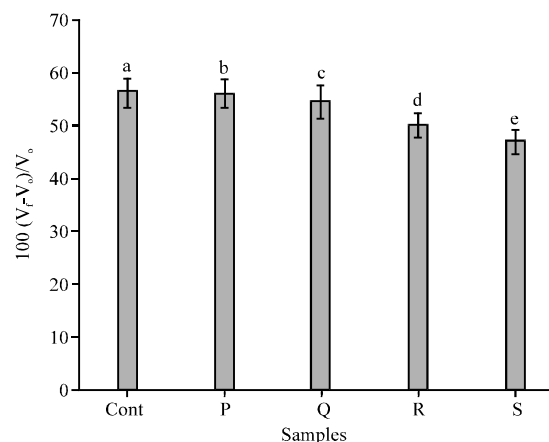


Fig. 1: Effect of percentage variation of wheatgrass on volume of idli batter at $p \leq 0.05$. Control idli is designated as ‘CONT’ and the fortified idlis are designated as ‘P’ (Idli with 0.5% wheatgrass powder), ‘Q’ (Idli with 1% wheatgrass powder), ‘R’ (Idli with 2% wheatgrass powder) and ‘S’ (Idli with 3% wheatgrass powder). Where V_f = Final volume, V_o = Initial volume

the sample S with 3% wheatgrass substitution shows 47%. Hence, the maximum decrease in volume is lesser than 10%. Idli batter is foam in which gas molecules are entrapped in a solid-liquid phase. The surface-active proteins from black gram act as surface-active agent and the polysaccharide acts as stabilizing agent (Damodaran and Paraf, 1995).

Increased level of wheatgrass powder disturbs the foam formation by means of decrystallization and degelatinization. Increased volume of idli batter is due to the incorporation of lactic acid bacteria into the batter during fermentation and entrapment of air (Ghosh and Chattopadhyay, 2011). In the study phenolics and flavonoids of wheatgrass (Das *et al.*, 2011) interacts with lactic acid bacteria and lowers the amount of total lactic acid bacteria in the batter, so as the percentage of wheatgrass increases, volume of the batter decreases.

pH measurement and total lactic acid concentration: The pH of the batter was observed (Table 1) to increase with the increase in the substitution level with wheatgrass powder. The highest pH is in the batter made from wheatgrass powder at 3% level of substitution. The total lactic acid content varies from 0.619-0.711%. pH and the acidity is directly related with total number of lactic acid bacteria present in the batter. Initially, it is same for all samples but wheatgrass powder acts as antimicrobial agent (Das *et al.*, 2012).

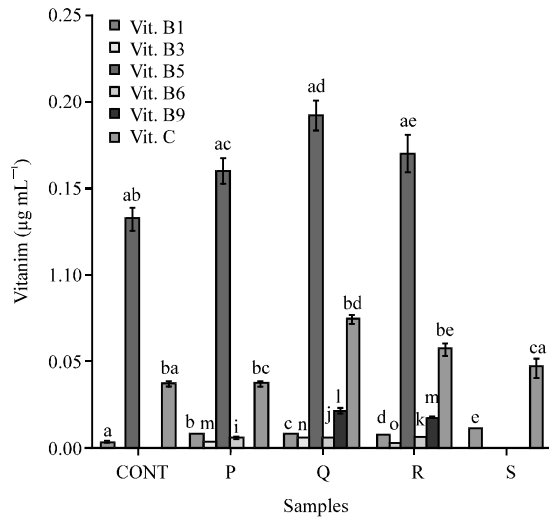


Fig. 2: Effect of percentage variation of wheatgrass on vitamin C and vitamin B profile of idli batter at $p \leq 0.05$. Control idli is designated as 'CONT' and the fortified Idlis are designated as 'P' (Idli with 0.5% wheatgrass powder), 'Q' (Idli with 1% wheatgrass powder), 'R' (Idli with 2% wheatgrass powder) and 'S' (Idli with 3% wheatgrass powder)

Table 1: Effect of different proportion of wheatgrass on pH and lactic acid (%) of idli batter

Samples	pH of idli batter	Lactic acid (%) of idli batter
CONT	5.00±0.003 ^a	0.711±0.012 ^a
P	5.15±0.01 ^b	0.703±0.030 ^b
Q	5.75±0.06 ^c	0.646±0.021 ^c
R	5.81±0.03 ^{ab}	0.631±0.010 ^{ab}
S	5.88±0.05 ^{bc}	0.619±0.026 ^{bc}

The control idli is designated as 'CONT' and the fortified idli are designated as 'P' (Idli with 0.5% wheatgrass powder), 'Q' (Idli with 1% wheatgrass powder), 'R' (Idli with 2% wheatgrass powder) and 'S' (Idli with 3% wheatgrass powder)

Vitamin estimation: Figure 2 shows the concentration of water-soluble vitamins (ascorbic acid, niacin, panthothenic acid (vitamin B₅), pyridoxine (vitamin B₆), thiamine (vitamin B₁) and folic acid) after 20 h of fermentation in different test samples. It is evident from the graph that sample Q provides maximum production of all vitamins.

Desikachar *et al.* (1965) found no change in thiamine content during fermentation of idli, Ghosh and Chattopadhyay (2011) found enhanced amount of vitamin B production during fermentation and 7 h is the recommended time for the production of maximum amount of B vitamins, possibly because they use *L. mesenteroides* whereas in the study the organisms involved were *Leuconostoc mesenteroides* (NCIM 2073) and *Lactobacillus plantarum* (NCIM 2084) and wheatgrass powder was used as prebiotic element.

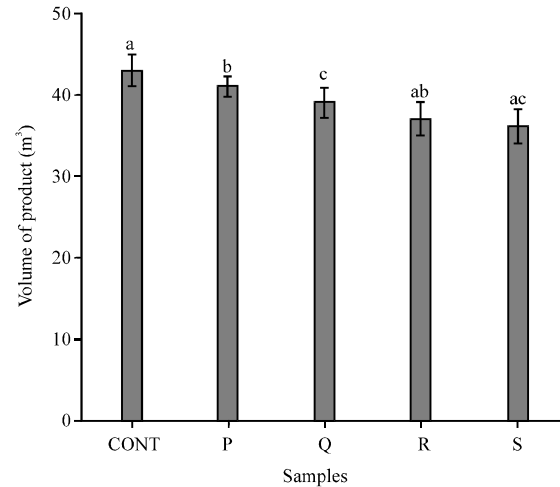


Fig. 3: Effect of percentage variation of wheatgrass on volume of idli at $p \leq 0.05$. Control idli is designated as 'CONT' and the fortified idlis are designated as 'P' (Idli with 0.5% wheatgrass powder), 'Q' (Idli with 1% wheatgrass powder), 'R' (Idli with 2% wheatgrass powder) and 'S' (Idli with 3% wheatgrass powder)

Effect of percentage variation of wheatgrass on the quality parameters of idli

Volume of idli: According to Fig. 3, control sample has highest volume (43 m³) whereas sample S shows lowest volume (36 m³). The specific volume shows a decreasing trend on increasing the level of wheatgrass powder supplement as compared with the control. It was measured by seed displacement method using mustard seeds. Volume of idli is directly proportional to the increase in volume of its batter.

Texture analysis of the idli samples: The texture of prepared idli was measured instrumentally. Hardness of a food sample may be defined as the force necessary to produce a given deformation; it is the peak force of the first compression of the product. Figure 4 showed that the control has the least hardness value (16.5 N) which is gradually increasing significantly ($p \leq 0.05$) with the increase in proportion of the wheatgrass powder.

Hardness of the product is indirectly proportional to the batter volume and volume of the product. Lesser degree of fermentation is responsible for increased hardness. As seen from the Fig. 4, there is maximum increase of hardness to a value (28.5 N) for the sample S from the value of (16.5 N) of the control sample.

Visual color parameters: The color of the idli is a characteristic first perceived by the consumer and affects the acceptability of the product. Table 2 shows the effect

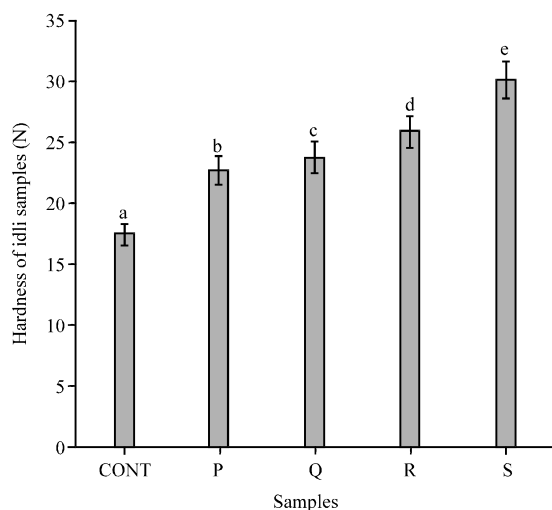


Fig. 4: Studies on hardness of idli fortifying with different agents at $p \leq 0.05$. Control idli is designated as 'CONT' and the fortified idlis are designated as 'P' (Idli with 0.5% wheatgrass powder), 'Q' (Idli with 1% wheatgrass powder), 'R' (Idli with 2% wheatgrass powder) and 'S' (Idli with 3% wheatgrass powder)

Table 2: Effect of addition of different proportion of wheatgrass on visual color of idli at $p \leq 0.05$

Samples	b/a	Hue angle
CONT	36.47 ± 0.010^a	88.42 ± 0.020^a
P	-20.15 ± 0.013^b	87.15 ± 0.024^b
Q	-12.15 ± 0.021^c	85.36 ± 0.015^c
R	-9.12 ± 0.010^d	83.74 ± 0.060^{ab}
S	-7.99 ± 0.025^e	82.86 ± 0.040^e

of addition of different percentage of fortifying ingredients on the visual color of idli. The browning for idli can be explained by higher hue angle and "b/a" values. Hue value of control is 88.42, hue values decreased with wheatgrass addition which suggested reduction from a more green (when Hue $> 90^\circ$) to an orange-red (when Hue $< 90^\circ$) colour (Waliszewski *et al.*, 1999) of cooked idli. Addition of fortifying agent lowers the browning of idli.

Antioxidant analysis: Figure 5 examines the correlation between the phytonutrient concentration and total antioxidant activity. Phenolic and flavonoid compounds are plant constituents that possess antioxidant activity and prevent the decomposition of hydroperoxidase in free radicals. Wheatgrass is a rich source of antioxidative compounds (Das *et al.*, 2011). The total phenolics, flavonoids content of fortified product and scavenging activity toward DPPH radical were measured. A better

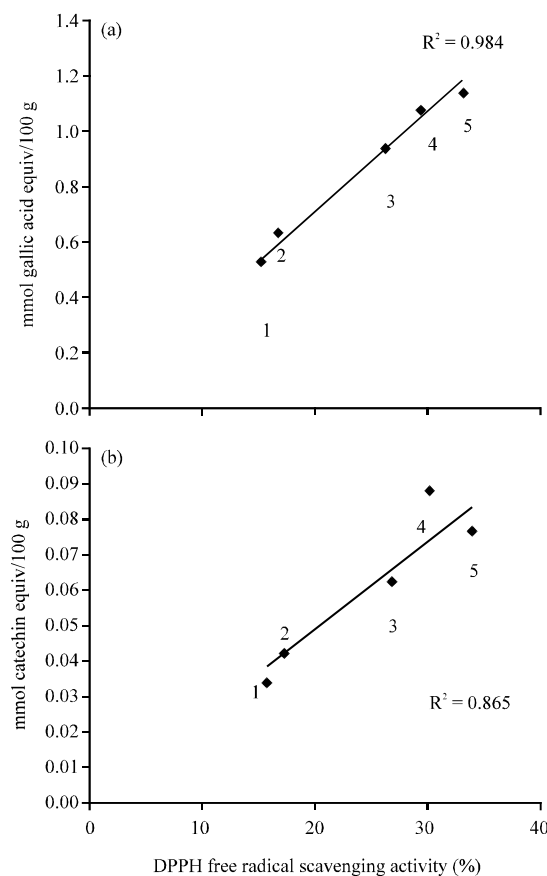


Fig. 5: Correlation of DPPH vs. TPC and DPPH vs. TFC values of methanolic dry extracts of idli from (1) control idli, (2) Idli with 0.5% wheatgrass powder, (3) Idli with 1% wheatgrass powder, (4) Idli with 2% wheatgrass powder, (5) Idli with 3% wheatgrass powder

correlation ratio was found in case of phenolics ($R^2=0.984$) than flavonoids ($R^2=0.865$) when analysed with respect to DPPH assay. It can also be inferred that the phenolic compounds are predominantly responsible for the antioxidant activities in the wheatgrass fortified idli samples.

Sensory analysis: Mean sensory scores obtained for various quality parameters of idlis are shown in the Fig. 6. The appearance of all the five samples differs from each other; the score range is between 5.1-8.2 which mean neither like nor dislike to like very much. Product containing 0-1% wheatgrass were judged to be significantly ($p < 0.05$) more acceptable than sample containing 2-3% wheatgrass. The score of taste is higher in case of sample Q. In terms of overall acceptability,

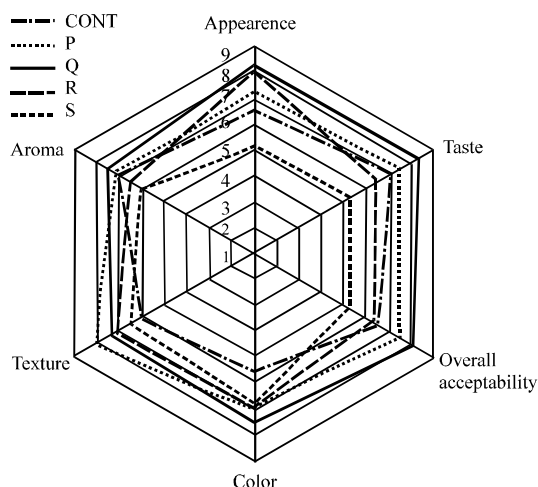


Fig. 6: Studies on sensory quality of idli. Control idli is designated as 'CONT' and the fortified idlis are designated as 'P' (Idli with 0.5% wheatgrass powder), 'Q' (Idli with 1% wheatgrass powder), 'R' (Idli with 2% wheatgrass powder) and 'S' (Idli with 3% wheatgrass powder)

sample Q got the highest score which comes under the grade of 'like very much' (points 8-9) in a 9 point hedonic scale.

CONCLUSION

The data presented in this study show the incorporation of wheatgrass powder in idli batter and their effect on nutritional, nutritional, textural, total antioxidant and sensory characteristics. It was found that addition of wheatgrass significantly affect the batter volume, pH, acidity, volume of the product, hardness and antioxidant activity. Very little effect was observed on visual color. Enhanced amount of B vitamins and vitamin C was found in 1-2% wheatgrass fortified products. The taste panel acceptability score showed that wheatgrass powder could be added up to one percentage.

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