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Title of paper	Name of the author/s	Department of the teacher	Name of journal	Year of publication	ISSN number
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The Antioxidant Efficacy of Wheatgrass (Triticum aestivum) on Mercuric Chloride (HgCl2) - Induced Oxidative Stress in Rat Model	Dr. Renu Tripathi	Home Science	Current Research in Nutrition and Food Science	2021	2347-467X
Effects of Thymol Supplementation Against Type 2 Diabetes in Streptozotocin- Induced Rat Model	Dr. Renu Tripathi	Home Science	Plant Archives	2020	0972-5210
A Study of Satisfaction Level of Farmers Form Cooperative Society (With Special Reference to Tribal Block Dondi in Balod District, C.G.)	Dr. Lalee Sharma	Commerce	Shodh Sanchar	2021	2229-3620
A Study of Satisfaction Level Form Banking Facilitiy Provided by Women Cooperative Bank to Its Members.	Dr. Lalee Sharma	Commerce	Shodh Sarita	2021	2348-2397

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Physicochemical and Antioxidant Potential of Garlic: Heat Processing Effects

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Abstract—*Heat processing method of fruits and vegetables has been developed over the centuries to make the final product more attractive in flavour, appearance, taste and consistency. Besides consumer preferences, the selected heat processing methods for food preparation is an important factor affecting not only the nutritional composition, but also the intake of bioactive compounds under normal dietary conditions. In this study garlic was evaluated for their, physicochemical, and antioxidant potential. The antioxidant content was significantly affected on application of different processing methods and processing time. Roasted sample exhibited higher % DPPH and total phenolic content as compared to the other heat processing methods. One of the roasting methods, microwave processing retained maximum ascorbic acid content, percent acidity and pH value. Hence roasting and microwave treatment may be recommended over other heat processing methods for better retention of bioactive compounds.*

Keywords*: Antioxidant potential, Garlic, Heat treatment, Physicochemical potential.*

1. INTRODUCTION

Antioxidant compounds in food are observed to possess health-protecting effects. Primary sources of natural antioxidants are whole grains, fruits and vegetables. Garlic (*Allium sativum*) has been used in world cuisines as well as in herbal medicine for thousands of years and has been claimed to prevent several diseases including cancer. Garlic (*Allium sativum*) is widely used in many parts of the world as vegetables as well as folk and modern medicine. In recent years garlic has received considerable attention in research for its pharmacological potential, which include lipid-lowering effects, anticancer activity, antioxidant activity, anti atherosclerotic activity, antimicrobial activity [1,2,3]. The cardiovascular effects of garlic are among the best investigated of all medicinal plants species [4]. Garlic contains minerals: calcium, copper, iron, manganese, phosphorus, potassium, selenium and Vitamins, A, B and C. Garlic contains various bioactive compounds including Allicin and its derivatives [5]. Garlic has also been proposed as one of the richest sources of total phenolic compounds among the generally consumed vegetables, and highly appreciated regarding its contribution of phenolic compounds in human diet.

Despite several therapeutic potential, raw garlic could not be consumed in large quantity due to its unpleasant taste and odour. Therefore, it is cooked as spice to make the final product more attractive in flavour, appearance, taste and consistency. Heat treatment, one of the most widely used post harvest processing method, is supposed to cause various physicochemical changes, including changes in flavor, colour and nutrient content. Heat treatment also leads to non-enzymatic browning reactions such as the Millard reaction, caramelization and chemical oxidation of phenols [6]. This study therefore has been planned to explore the effect of various heat processing methods as boiling, roasting, frying, steaming and microwaving on physico-chemical attributes and, consequently bioactive potential of garlic.

2. MATERIALS AND METHODS

2.1 Materials

Garlic was procured from the local market, cleaned removed extraneous materials and debris; edible bulbs were collected for further analysis. All standard chemicals were obtained from Sigma Chemicals (USA). Other chemicals, reagents, solvents used in this study were of analytical, extra pure grade.

3. METHODS

3.1 Heat processing methods

Five heat processing methods viz., boiling, steaming, frying, roasting and microwaving were selected for the study. These methods were proceeded for 5, 10, and 15 minutes respectively. All these experiments were performed in triplicate, each using 100 g of garlic sample.

3.2 Boiling

About 200 ml of distilled water was poured into a 500 ml beaker and put on the hot plate (Bezij 15090012008, India). As the water began to boil, the chopped garlic sample (100g) was added and boiled for 5, 10, 15 minutes respectively. After that sample was drained off and cooled [7].

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3.3 Roasting

100 g of crushed garlic sample was roasted in tray drier (Science tech. India) at 190º C for 5, 10, and 15 min. After roasting, the roasted garlic sample was collected and cooled [8].

3.4 Frying

100 ml of soybean oil was placed in a frying pan (30 cm in diameter) and heated on a hot plate for 1 min. 100 g of garlic sample was then placed in the pan, and the heating was reduced to medium and fried for 5, 10, and 15 min. Next, the fried garlic was drained off and cooled [9].

3.5 Steaming

100 g of crushed garlic sample was placed on a tray in a stainless steel steam cooker, which was covered with a lid, and steamed over 95ºC, for 5, 10, and 15 min under atmospheric pressure. After steaming, the garlic was drained for 1 min using a wire mesh strainer and then cooled [7].

3.6 Microwaving

Garlic (100g) was added to 200 ml of distilled water in a 500 ml beaker and then cooked in a domestic microwave oven (Whirlpool 021128620) for 5, 10, 15 min. The beaker was covered with watch glass to prevent water loss. The sample was drained off and cooled [10].

4. PHYSICOCHEMICAL ANALYSIS OF FRESH AND PROCESSED GARLIC

4.1 Determination of Moisture content

The moisture content of garlic was determined using standard method of AOAC [11]. Dishes used for the moisture determination were first dried at 105°C for 1 hour in drying oven. It was then transferred to the desiccators, cooled for 30 minutes, and weighed. The prepared samples were mixed thoroughly and about 5 g of the samples were transferred to the dried and weighed dishes. The dishes and their contents were placed in the drying oven and dried for 3 hrs at 105°C, and then the dishes and their contents were cooled in desiccators to room temperature and reweighed.

4.2 Determination of pH

10g garlic paste was homogenized with 50ml-distilled water in a mixer grinder. The ground sample was filtered and the pH was determined by dipping the combined glass electrode of a digital pH meter (Khera model, Indian make) into the filtrate.

4.3 Determination of percent acidity

Acidity of garlic was determined using the method of Ranganna [12]. To prepare the sample, 10 gm sample was boiled in 100 ml of distilled water for one hour, replacing the water lost by evaporation. It was then cooled, filtered and transferred to a volumetric flask and made up to 100 ml with distilled water. 10 ml of the aliquot was pipette out and titrated with 0.1N NaOH using few drops of phenolphthalein as indicator. The titre value was noted and percent acidity was calculated using the following equation:

$$\% \text{ Acidity} = \frac{\text{Titre x Normality of NaOH x Volume made up x Eq.wt. of Citric Acid x 100}}{\text{Volume of Sample taken for Estimation x wt. or volume of sample taken x 1000}} \times 100$$

4.4 Determination of color

Color determination was carried out using the Hunter *L*, *a*, *b* system with a colorimeter (Minolta CR-300). Both fresh and processed samples were analyzed. The assessments were carried out at room temperature (25ºC). *L** values indicate white to dark (lightness, black = 0, white = 100), *a** values green to red (redness > 0, greenness < 0), and *b** values blue to yellow (yellowness, b* > 0, blue < 0). The individual differences in L*,a*and b*values of each heat treatment with respect to the color of the fresh sample was evaluated using ΔE calculation [13]. The total color difference (Δ*E*) was calculated using the following equation:

$$\Delta E = (\Delta L^{2} + \Delta a^{2} + \Delta b^{2})^{1/2}$$

5. ANALYSIS OF ANTIOXIDANT POTENTIAL OF FRESH AND PROCESSED GARLIC

5.1 Total polyphenol content

The total phenolic content of extract was determined using the Folin-Ciocalteu reagent according to modified method described by Singleton *et al.* [14]. Standard solution or extract (0.2 ml) was mixed with 2 ml of 2% Na_2CO_3 solution and 0.1 ml of 50% Folin-Ciocalteu reagent. After 30 min, the absorbance was read at 750 nm, and TPC was calculated from a calibration curve that was obtained using gallic acid as the standard. The results were expressed as milligrams of gallic acid equivalents per 100 g fresh weight. Extract was analyzed in triplicates.

5.2 DPPH radical scavenging activity

The free radical scavenging activity of the extract was measured using DPPH (1, 1- diphenyl 2-picryl hydrazyl) method of Shimada *et al.* [15] with slight modification. 10 mg of crushed garlic sample was mixed with 10 ml acidified methanol and heated at 40°C in water bath for 30 min. 100 µl of sample extract thus prepared was kept in a test tube and diluted with 2.9 ml of pure methanol. Sample was mixed with 150 µl of DPPH solution, incubated for 15 min. in dark and absorbance was measured in UV visible spectrophotometer at 515 nm. The % radical scavenging activity was calculated using following formula:

Control Absorbance-Sample Absorbance/Control Absorbance × 100

5.3 Determination of Ascorbic acid

Ascorbic acid content was estimated by standard A.O.A.C [11] method. 2g sample was crushed in Meta phosphoric acid solution and volume was made up to 50 ml in volumetric flask.15 g Meta phosphoric acid added in 40 ml acetic acid containing 200 ml distilled water. Standard ascorbic acid solution of 1mg/ml was made.

6. STATISTICAL ANALYSIS

The results were reported as mean ± standard deviation (SD) values. The significant differences among the means were determined with one way analysis of variance (ANOVA) using IBM SPSS statistics version 20 at a significance level of 0.05.

7. RESULTS

7.1 Effect of processing methods on moisture content

The moisture content of fresh and processed garlic was presented in Fig. 1. The moisture content of garlic was significantly ($p<0.05$) affected by boiling, steaming, frying, microwaving and roasting. The moisture content of fresh garlic was 65.76 mg/100g. It varied from 41.5 to 83.72 mg/100g (dwb).

Moisture content of fresh and processed garlic

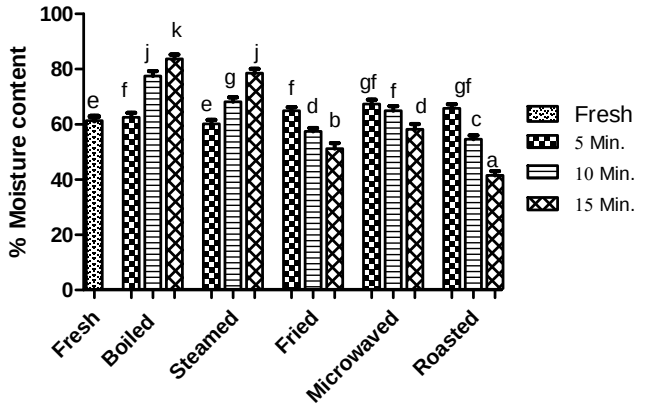

Fig. 1: Effect of processing methods and processing time on the moisture content of garlic. Mean values on the bars with different letters are significantly different at p<0.05.

7.2 Effect of processing methods on pH value

The garlic sample was treated with boiling, steaming, frying, roasting and microwave processing, and the pH value of the sample was examined. Changes in pH at various temperatures are shown in Fig. 2. The pH of fresh garlic was 6.54, whereas after heat treatments boiling, steaming, frying, microwave and roasting, the pH value of the garlic sample was 5.26, 5.13, 4.89, 4.99 and 4.49 respectively.

pH Value of fresh and processed garlic

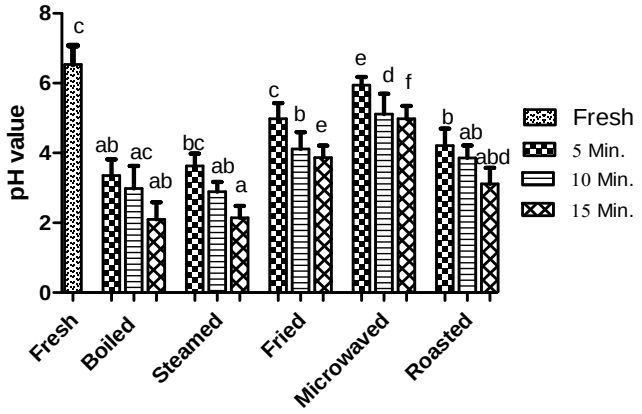

Fig. 2: Effect of processing methods and processing time on the pH value of garlic. Mean values on the bars with different letters are significantly different at p<0.05.

7.3 Effect of processing methods on percent acidity

In investigating the effects of heat treatment on the percent acidity of garlic, it was revealed that the percent acidity decreased as the processing time increased. In fresh garlic, percent acidity was 1.94 %. For boiling, steaming, frying, roasting, and microwaving, the percent acidity decreased by 0.12～0.37, 0.31～0.38, 1.21～1.32, 1.45～1.62 and 1.75～1.86 % respectively Fig. 3.

% Acidity of fresh and processed garlic

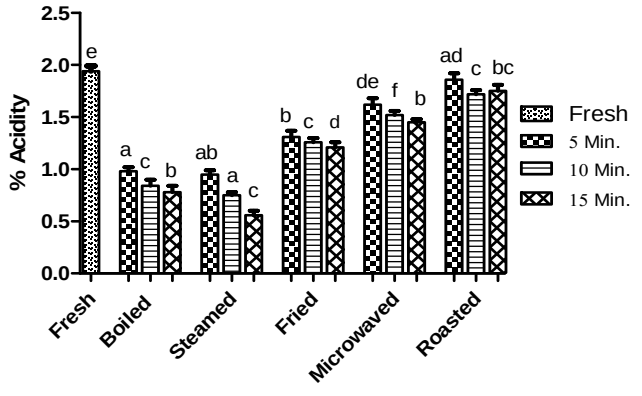

Fig. 3: Effect of processing methods and processing time on the percent acidity of garlic. Mean values on the bars with different letters are significantly different at p<0.05.

7.4 Effect of processing methods on color value

The Hunter color L*, a* and b* values of fresh garlic were 62.84, -1.97 and 15.75, respectively. Effect of the processing

methods and time on the Hunter color value is shown in Table 1. Lower L* value in processed garlic reveals that after various type of processing the brightness of sample was less as compared to fresh samples. With the increase in the processing time the L* value decreased further. a* value of samples increased after processing which indicates that the sample redness increased after processing. b* value increased after roasting, frying and microwave treatment.

Table 1: Colour value of the fresh and processed garlic during different processing method and processing time.

Heat Treatments		L*	a*	b*	ΔE
Fresh		62.84 ±1.61^{de}	-1.97± 0.53^{bc}	15.75±0.35^{c}	-
Boiling	5 min	60.35±1.46^{d}	-2.37±0.09^{d}	11.05±1.24^{b}	5.33^{a}
	10 min	58.21±1.28^{cd}	-2.29±0.06^{a}	10.25±1.41^{b}	7.19^{b}
	15 min	57.05±1.83^{bcd}	-2.20±0.13^{a}	9.64±0.10^{ab}	8.42^{cd}
Steaming	5 min	54.42±1.34^{ab}	-1.58±0.02^{ad}	10.35±0.04^{b}	10.01^{efg}
	10 min	48.41±1.42^{a}	-1.98±0.52^{ab}	9.35±0.05^{ab}	15.78^{i}
	15 min	43.41±1.54^{abc}	-2.86±0.68^{a}	8.35±0.05^{d}	20.81^{hi}
Frying	5 min	50.14±1.46^{abc}	2.62±0.06^{cd}	20.08±0.34^{a}	14.18^{j}
	10min	49.56±1.35^{ab}	3.45±0.06^{def}	19.25±1.41^{d}	14.76^{hi}
	15 min	48.2±0.36^{de}	4.47±0.47^{fg}	18.82±0.89^{d}	16.28^{i}
Microwaving	5 min	61.29±0.35^{e}	1.15±0.89^{cd}	24.88±0.45^{a}	9.77^{fe}
	10 min	59.28±0.29^{cd}	1.26±0.69^{de}	22.24±1.25^{efg}	7.83^{cd}
	15 min	58.29±0.43^{d}	1.35±0.58^{defg}	20.45±1.36^{d}	7.33^{c}
Roasting	5 min	60.29±0.38^{b}	1.78±0.05^{cde}	26.46±1.42^{g}	11.63^{g}
	10 min	59.19±0.25^{cd}	1.88±0.05^{efg}	24.65±1.34^{f}	10.37^{fg}
	15 min	57.66±0.85^{cd}	1.95±0.72^{g}	22.5±0.12^{e}	9.36^{def}

Values are expressed as mean ± standard deviation of triplicate experiment. Mean values in a column with different letters are significantly different at p< 0.05.

7.5 Effect of processing methods on total phenolic content

The Total Phenolic Content in fresh and processed garlic, expressed as milligrams of gallic acid equivalent per 100 g of fresh weight, is presented in Fig. 4. The TPC in fresh garlic was 78.45 mg/100 g. For boiling, steaming, frying, microwaving, and roasting, TPC decreased by 34.18～52.87, 59.12～62.59, 69.84～72.43, 71.25～75.25 and 74.39～76.67 mg/100 g respectively.

Total Phenolic Content of fresh and processed garlic

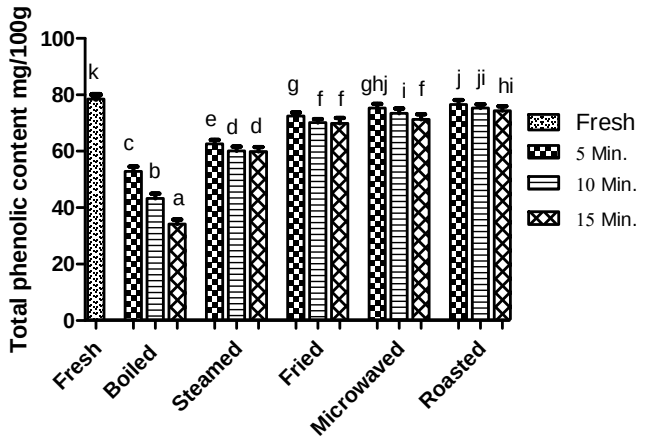

Fig. 4: Effect of processing methods and processing time on the Total Phenolic Content of garlic. Mean values on the bars with different letters are significantly different at p<0.05.

7.6 Effect of processing methods on DPPH radical scavenging activity

The DPPH radical scavenging activity of fresh and processed garlic, expressed as milligrams of AA equivalent per 100 g of fresh weight, is presented in Fig. 5. The DPPH radical scavenging activity of garlic extract was significantly reduced (p<0.05) after processing.

DPPH Radical Scavenging Activity of fresh and processed garlic

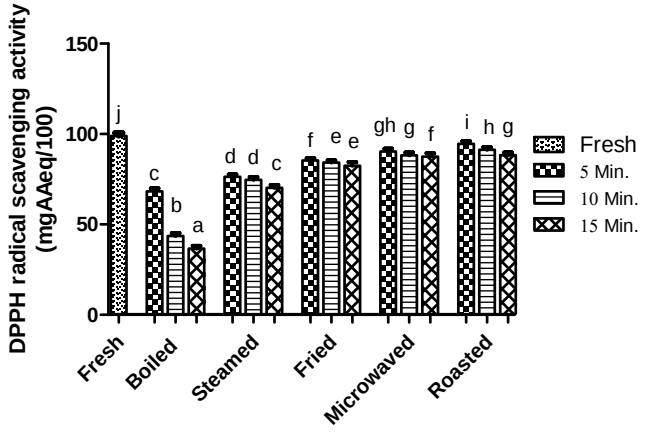

Fig. 5: Effect of processing methods and processing time on the DPPH radical scavenging activity of garlic. Mean values on the bars with different letters are significantly different at p<0.05.

The DPPH radical scavenging activity of fresh garlic was 98.92 mg AA eq/100 g and after processing, decreased by 36.55～68.29, 70.15～76.30, 82.45～85.35, 87.48～90.36 and 88.28～94.56 mg AA eq/100 g for boiling, steaming, frying, microwaving and roasting respectively.

7.7 Effect of processing methods on ascorbic acid content

The Ascorbic Acid levels of fresh and processed garlic are presented in Fig.6. The Ascorbic acid content of fresh garlic was 27.53 mg/100 g. All processing methods lead to a significant loss ($p<0.05$) in the amount of Ascorbic acid as compared to fresh garlic. As processing time increased, the Ascorbic acid content of boiled, steamed, fried, microwave and roasted garlic decreased, with a range of 10.40～12.26, 15.68～18.51, 19.48～22.64, 21.46～23.48 and 17.54～20.24 mg/100 g, respectively. For boiling, steaming, frying, microwaving, and roasting, the mean Ascorbic acid content lost after for 15 min were 55.4%, 32.7%, 26.4, 17.7%, and 14.7% respectively. The result of the effect of five different heat-processing methods indicated that the highest reduction was noted after boiling, followed by steaming, roasting, frying and microwaving.

Ascorbic acid content of fresh and processed garlic

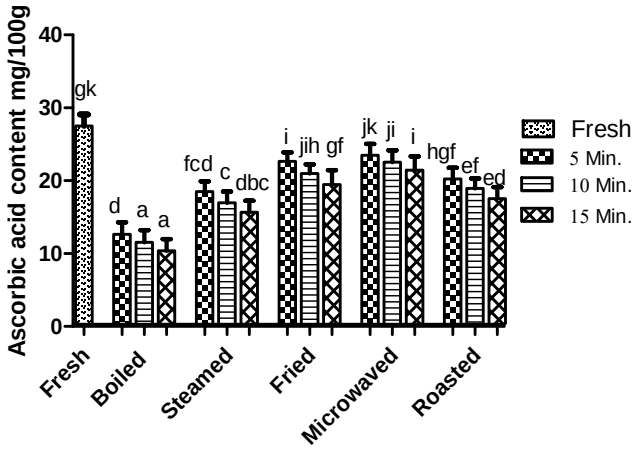

Fig. 6: Effect of processing methods and processing time on the ascorbic acid content of garlic. Mean values on the bars with different letters are significantly different at $p<0.05$.

8. DISCUSSION

Changes eminent in the moisture content of the garlic is due to the application of various heat treatments. During the dry heat treatment process (roasting, frying and microwaving), lower moisture contents were at higher temperatures [16] whereas during moist heat treatment process i.e. boiling and steaming moisture content were increased continuously over time. Boiling has higher moisture content due to the absorption of water during processing.

It was found that the pH value of five minutes microwave treated sample decreased by lesser amounts than that of other heat-treated samples. A significantly decrease ($p<0.05$) in pH value was observed with the progression of heat treatment process. Thereafter, the pH decreased gradually with increased temperature. Similar results were also observed in the red ginseng manufacturing process, and the decrease in pH progressed as the heating temperature increased [17].The pH decrease in the heated garlic sample was, in part associated with the production of browning materials during heat treatment. The formation of carboxylic acids (which are produced by the oxidation of aldehyde group in aldohexose, acidic compounds and decrease in basic amino acid by combing with sugar) has been reported responsible for the decrease in pH, in browning reaction and during heat treatment.

All the five heat processing methods i.e. boiling, steaming frying, microwaving and roasting significantly ($p<0.05$) affected the percent acidity in garlic compared with fresh garlic. The highest reduction was observed after boiling (80.9～93.8%), followed by steaming (80.8～84.0%), frying (31.9～37.6%), microwaving, (5.6～9.7%) and roasting (16.4～25.2%), whereas maximum retention was observed in microwaving (5.6～9.7%). Similarly the mean acidity of boiled vegetables was found to be decreased with increased boiling time [18].

The change in color calculated from color parameters ‘L’ ‘a’ ‘b’ of fresh and processed samples in the range of 0.10 to 1.35 reveals, an imperceptible color difference.

Dry heat processing methods i.e. frying, microwaving and roasting did not significantly ($p<0.05$) affect the total phenolic content in processed garlic when compared to fresh garlic, whereas boiling and steaming significantly decreased ($p<0.05$) total phenolic content. The highest reduction was observed after boiling (32.60～56.4%), followed by steaming (20.2～24.6%), frying (7.6～10.9%), microwaving, (9.1～4.0%) and roasting (5.1 ～2.3%). Similar kind of result was obtained by Chuah *et al.* [19] and found that boiling significantly reduced the total phenolic content in colored peppers, while fry and microwave cooking had no impact on total phenolic content. Zhang [10] and Ismail *et al.* [20] reported that cooking vegetables significantly decreases, total phenolic content. Reduced phenolic compounds in boiled or steamed foods have been attributed to the dissolution of phenolic compounds into the cooking water [21]. The loss of phenolic compounds also depend on the processing time and food size [22]. In contrast, other studies found that cooking increased Total phenolic contents in some vegetables due to the disruption of cell walls, which liberated soluble phenolic compounds from insoluble ester bonds [23,24].

The DPPH radical scavenging activity was reduced by 30.9～63.0% of its initial capacities after boiling, followed by steaming (22.8～29.0%), frying (13.7～16.6%), microwaving (8.6～11.5%) and roasting (4.4～10.7%).

The results show that moist-heat processing methods i.e. boiling and steaming result in high losses of ascorbic acid, while dry heat processing methods i.e. frying, roasting and microwave treated sample revealed only small losses. Various studies have shown that cooking reduces Ascorbic acid content in fruits and vegetables; therefore, as a commonly perceived idea, Ascorbic acid is destroyed during heat processing because it is unstable at high temperature. Chuah *et al.* [25] reported that the Ascorbic acid levels in peppers decreased during cooking procedures such as boiling, microwave cooking, and stir-frying. Significant reductions were documented for boiling particularly due to the diffusion of Ascorbic acid into cooking water. Somsub *et al.,* [26]**,** and Leskova *et al.* [27] reported higher Ascorbic acid retention values in foods processed by stir frying, roasting and microwave cooking than in those processed by boiling or blanching. Various studies report that cooking reduces Ascorbic acid content in food, including sweet chestnuts, potatoes, tropical leafy vegetables, selected Thai vegetables, broccoli and sweet peppers [28,29]. The amount of cooking related loss of Ascorbic acid depends on several factors, including cooking method, heating temperature, cooking time, enzymatic oxidation during preparation, and surface area exposed to water and oxygen.

9. CONCLUSION

It has been observed that change in physiochemical and antioxidant potential depends on type of heat processing methods used. Antioxidant potential was significantly affected on the application of different processing methods and time. Roasted samples exhibited higher total phenolic content and radical scavenging activity as compared to the other heat processing treatments. Ascorbic acid content, percent acidity and pH value retained better in microwave processed sample. Therefore, mild roasting and microwave treatment may be considered better and healthy processing treatment over others.

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RESEARCH ARTICLE

DEVELOPMENT AND OPTIMIZATION OF VALUE ADDED BREAD USING RESPONSE SURFACE METHODOLOGY

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ABSTRACT

Natural antioxidants or phytochemical antioxidants are secondary metabolites of plants. Ginger (*Zingiber officinale*) can be a major source of natural or phytochemical antioxidants such as beta-carotene, ascorbic acid, terpenoids, alkaloids, and polyphenols such as flavonoids, flavones glycosides, rutin etc. It is an important commercial crop which is, used both as spice and medicine and its therapeutic benefits of ginger are mainly due to the presence of volatile oils. Consumption of bakery products in the country is increasing day by day and bread is a staple food having several attractive features. In the view of health benefit of ginger it may be worthwhile to explore possibility of incorporating ginger extract in wheat flour for the development of bread to provide a convenient food to supplement the diet.. Present study was an effort to standardize the level of ginger extract in formulation for the development of value added bread. To optimize the quantity of sugar and ginger extract to be added, Response Surface Methodology (RSM) was used, while rest of the ingredient level was kept constant. The lower and upper limits for sugar and ginger extract were taken as 20-30 g and 10-20 ml, respectively. Control treatment was prepared without ginger extract addition. All thirteen combinations and control were subjected for sensory quality evaluation on a 9 point hedonic scale. From the study, it was found that the bread having composition 17.93 g sugar, and 15 ml ginger extract per 100 g of bread was found optimum and the said formulation was acceptable and recommended for value added bread. The optimized bread was found to be superior in terms of minerals, calcium and iron as compared to control bread. Since, the bread was a good source of calcium and iron, hence it can be recommended for consumption for children and old age people. The addition of ginger extract, also gave an excellent antioxidant effect on the bread compared with control. It can be recommended as one of the value added products.

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INTRODUCTION

Ginger *(Zingiber officinale)* is an important commercial crop belonging to the family Zingiberaceae grown for its aromatic rhizomes, which are used both as spice and medicine (Badreldin *et al*., 2008). It can be used fresh, dried and powdered, or as a juice or oil. Ayurvedic medicine utilizes it for the treatment of arthritis (Thomson *et al*., 2002). Other traditional uses of ginger include colic, colds, fever, menstrual cramps and appetite stimulant (Chrubasik *et al*., 2005). Ginger supplements are widely available and include liquid extract, syrup, tea and capsules. The herbal therapeutic benefits of ginger are mainly due to the presence of volatile oils and the high oleoresin content. A compound known as gingerol (Bhattarai *et al*., 2001) is an acrid chemical constituent of the ginger, and this chemical compound is the agent responsible for the hot taste of ginger and is also one of the reasons that ginger possesses stimulating properties on the body (Wang *et al*., 2003). The aroma of ginger is pleasant and spicy and its flavor is penetrating, slightly biting due to antiseptic or pungent compounds present in it, which make it indispensable in the manufacture of a number of food products like ginger bread, confectionery, ginger ale, curry powder, certain soft drinks like cordials, ginger cocktail, carbonated drinks, bitters, etc. Ginger is also used for the manufacture of ginger oil, oleoresin, essences, tinctures, etc (Francisco *et al*., 2008). Bakery products are an important source of nutrients viz. energy, protein, iron, calcium and several vitamins. Most bakery products can easily be enriched and fortified at a low cost with proteins and various vitamins and minerals to meet the specific needs of the target groups and vulnerable sections of the population, who are undernourished and malnourished. Since fortification and enrichment can be easily carried out, it is very important that more nutritious products should be produced in future (Sharma *et al*., 2013). Bread is a staple food having several attractive features mentioned above and these

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features enhance the value of producing bread from composite flours for organoleptic, economic and nutritional reasons. In places where soft wheat flour is not available or too expensive, it becomes economically necessary to produce bread from composite flours. Nutritionally, bread can be easily fortified with ginger extract to provide a convenient food to supplement the diet for better nutrition. Since consumption of bakery products in the country is increasing day by day and in the view of above health benefit of ginger, it may be worthwhile to explore possibility of incorporating ginger extract in wheat flour. Present study was an effort to standardize the level of ginger extract in formulation for the development of value added bread.

MATERIALS AND METHODS

Ginger used for this investigation were purchased from the local market. All required ingredients like as sugar, refined white flour, dry yeast, water and common salt and refined oil for greasing agent were purchased from local market of Allahabad. India. All the chemicals used in analysis were of AR (analytical reagent) grade. This work was carried out at Centre of Food Technology, University of Allahabad, Allahabad, U.P., India.

Preparation of aqueous ginger extract

The ginger was washed, drained and then grated with the help of grater and then the aqueous extract was prepared (Puranik *et al*., 2013) to incorporate it in the bread preparation.

Development and Optimization of value added bread

Bread was prepared as per the slightly modified method (Nazni and Gracia, 2014). The ingredients such as refined wheat flour, sugar and salt were mixed for 1-2min. Then, yeast dissolved in lukewarm water (30-35ºC), which is the optimum temperature for the yeast cells to be activated, and finally the extracts was added to the dry ingredients. All the ingredients were again mixed for 2min and during mixing, water was added to the mixture. After mixing, the dough was kept for fermentation for 30 min. After that, the dough was placed in aluminium baking pan for proofing at an incubation chamber at 35 ºC and 80% relative humidity for 30 min. Then, the samples were ready for baking. Baking of sample was conducted in a laboratory oven with air circulation at (150 ºC) for 30 min. The loaves were removed from the pans and cooled at room temperature. To optimize the quantity of sugar and ginger extract to be added, Response Surface Methodology (RSM) was used while rest of the ingredient like refined white flour, dry yeast, water and common salt (Girdhari Lal *et al*., 2010) level was kept constant on the basis of hit and trial method using 9-point hedonicscale. Response Surface Methodology (RSM) is a collection of statistical and mathematical technique useful for developing, improving and optimization process (Mugwiza Telesphore and He, 2009), for statistical and graphical analysis of the experimental data and also for monitoring the combined effects of variables (Philip John Kanu *et al*., 2007). The sugar (17.93 g) and ginger extract (15ml) was repeated 5 times as central points. The lower and upper limits for sugar and ginger extract were taken as 20-30g and 10-20ml, respectively. Control treatment was prepared without ginger extract addition. All 13combinations and control were subjected for sensory quality evaluation by 15 trained panelists. The process flow chart is adopted for the preparation of bread by using various ingredients like refined white flour, dry yeast, water, refined oil and common salt. The process flow chart for the preparation of bread is given in Fig. 1.

Collection of raw material
↓
Proper mixing of ingredient with ginger extract and sugar
↓
Dough was prepared with yeast, water & kneaded
For 10-15 minutes
↓
Fermentation of dough for a period of 30 min
& covered with wet cloth
↓
Loaf was moulded and rest for 5-10 minutes
↓
Preheated and baked in oven at 150^{o}C for 30 minutes
↓
Cooled and sliced

Fig. 1. Process flow chart in the preparation of value added bread

Proximate and Mineral content analysis

The moisture, crude fat, fibre and protein content of the samples were determined as per AOAC (2005) procedures; whereas minerals (Ca and Fe) were estimated as per the AOAC (1990) procedures.

Sensory Analysis

To carry out initial optimization of the ingredients of RSM design, thirteen combinations were judged by a trained panel of 15-members using a 9 point hedonic scale (9-like extremely and 1-dislike extremely) (Murray *et al*., 2001) for color, flavor, texture and mouth feel.

Determination of antioxidant activity

The antioxidant activities of native and processed raw materials were also measured by the DPPH radical scavenging method (De - Ancos *et al*., 2002). An aliquot (0.10ml) of sample extract in methanol was mixed with 2 ml of methanolic 0.1 mM DPPH solution and the volume was made up to 5 ml with distilled water. The mixture was thoroughly vortex-mixed and kept in dark for 30 min. The absorbance was measured at 515 nm. The result was expressed as percentage of inhibition of the DPPH radical. The percentage of inhibition of the DPPH radical was calculated according to the following equation:

$$\% \text{ inhibition of DPPH} = ((\text{Abs control} - \text{Abs sample})/(\text{Abs control})) \times 100$$

where, Abs control is the absorbance of the DPPH solution without the extract.

Determination of Total Phenol Content

Total polyphenols were estimated as per procedure described by (Singleton *et al*., 2005) using folin ciocalteu method, where 250 mg sample was taken in 10 ml of acetone and water (70:30 v/v) solution in a graduated test tube and heated on water bath at 70^{o}C for 10 min. The sample was brought to room temperature, centrifuged at 3500 rpm for 10 min. The supernatant (0.2 ml) was made up to 10 ml with distilled water.

This solution was diluted 10 fold and sample solution (5 ml) was mixed with saturated sodium carbonate (0.5 ml) and Folin-Ciocalteaue reagent (0.2 ml) and made up to 10 ml with distilled water. The absorbance was read at 765 nm after 60 min by UV visible double beam spectrophotometer (Model Evolution 600, Thermo Electron, US).

Texture analysis of Optimized Bread

Hardness was measured by Texture analysis of control and optimized developed bread with the help of Texture analyzer by operating Force in compression, Pre test speed – 5mm/sec, Test speed – 10mm/sec, Post test speed-10mm/sec, distance - 8mm/sec and Load cell -5kg.

Statistical Analysis

The data obtained were analyzed statistically for analysis of variance (ANOVA) using completely randomized design with least significant difference (LSD) at $P < 0.05$ using Co. Stat 6.303, CoHort software (USA).

RESULTS AND DISCUSSION

Optimization of Value added bread

For the optimization of the variables, the responses colour, flavour, texture, mouth feel and overall acceptability were selected. From the ANOVA result in Table 2 there was significant difference found for each variable of sensory attributes at $p<0.05$. All these responses have been shown to create direct effect on the quality of bread (Puranik *et al*., 2013). Interaction between sugar and ginger extract showed positive ($P < 0.05$) effect on the flavour as we increase the concentration of ginger extract (Fig. 2). Interaction between sugar and ginger extract to colour had significant negative effect on colour ($P < 0.05$) (Fig. 3). The interactive effect of ginger to sugar showed positive effect on texture of bread (Fig. 4). On increasing the amount of ginger extract and sugar, mouth feel increases (Fig.5). Interaction between sugar and ginger extract showed positive ($P < 0.05$) effect on the overall acceptability of optimized bread (Fig 6). The overall effect of ginger extract and sugar was maximum on all sensory

Table 1. Effect of variables on the sensory attributes of developed ginger bread

Variables		Sensory Responses				
Ginger exract (ml)	Sugar(g)	Colour	Texture	Flavour	Mouth feel	Overall Acceptability
10.00	20.00	7.9	7.4	7.3	7.5	7.6
20.00	20.00	8.1	8.3	8.3	8.2	8.2
10.00	30.00	8.3	5.3	5.2	5.4	5.5
20.00	30.00	8.2	8.3	8.3	8.1	8.2
7.93	25.00	6.9	8.1	8.1	8.2	8.2
22.07	25.00	8.3	8.4	8.3	8.3	8.4
15.00	17.93	7.1	8.3	7.9	8.5	7.4
15.00	32.07	7.9	5.9	5.4	5.8	5.7
15.00	25.00	8.2	7.8	7.6	7.9	7.9
15.00	25.00	7.6	6.7	6.6	6.9	6.8
15.00	25.00	7.8	7.6	7.5	7.5	7.6
15.00	25.00	8.1	8.3	8.4	8.1	8.2
15.00	25.00	8.2	7.8	7.7	7.9	7.7
	Control	7.2	7.9	8.5	8.4	8.6

Table 2. ANOVA results for value added bread

Source	Color	Texture	Flavour	Mouthfeel	Overall acceptability
Model SS	0.69	8.46	10.04	8.03	8.48
Model DF	5	5	5	5	5
Mean MS	0.14	1.69	2.01	1.61	1.70
Pure Error	0.29	1.37	1.65	0.91	1.09
Mean	7.95	7.48	7.38	7.45	7.49
F cal.	0.90	4.08	4.00	4.91	5.29
F tab.	0.5289	0.0470	0.0493	0.0301	0.025
R square	0.3918	0.7444	0.7406	0.7781	0.7906

Table 3. Nutritional composition of Control and Optimized value added bread

Bread sample	Moisture (%)	Energy (kcal)	Protein (gm/100g)	CHO (gm/100g)	Fat (gm/100g)	Ash (gm/100g)	Ca (mg/100g)	Fe (mg/100g)	Fibre (gm/100)
Control N	34.5±0.28	258±0.57	6.4±0.05	56.49± 0.04	0.58±0.00	1.47±0.23	12.1±0.05	2.5±0.05	0.2±0.03
Optimised A	23.5±0.23	304±2.30	7.6±0.11	66±0.04	1.0±0.23	1.9±0.42	23±0.57	3.0±0.04	2.0±0.05

Table 3. Nutritional composition of Control and Optimized value added bread

Bread sample	Moisture (%)	Energy (kcal)	Protein (gm/100g)	CHO (gm/100g)	Fat (gm/100g)	Ash (gm/100g)	Ca (mg/100g)	Fe (mg/100g)	Fibre (gm/100)
Control N	34.5±0.28	258±0.57	6.4±0.05	56.49± 0.04	0.58±0.00	1.47±0.23	12.1±0.05	2.5±0.05	0.2±0.03
Optimised A	23.5±0.23	304±2.30	7.6±0.11	66±0.04	1.0±0.23	1.9±0.42	23±0.57	3.0±0.04	2.0±0.05

responses followed by control. To consider all the responses simultaneously for optimization, the RSM was used to compromise optimum conditions and it was found that the sensory scores were 7.1, 8.3, 7.9, 8.5 & 7.4 for colour, texture, flavor, mouth feel and overall acceptability corresponding to optimum conditions (Table 1).

Bread having composition 17.93 g sugar, & 15 ml ginger extract per 100 g bread was found optimum. Triplicate samples were prepared using the optimum conditions and were evaluated for all the responses. Corresponding values for color, texture, flavor, mouth feel and overall acceptability were 7.1, 8.3, 7.9, 8.5 & 7.4 respectively which were comparatively higher than the predicted value (Table 2). Therefore, bread having composition 17.93 g sugar, 15 ml ginger extract per 100 g of bread was found optimum and the said formulation was recommended for value added bread.

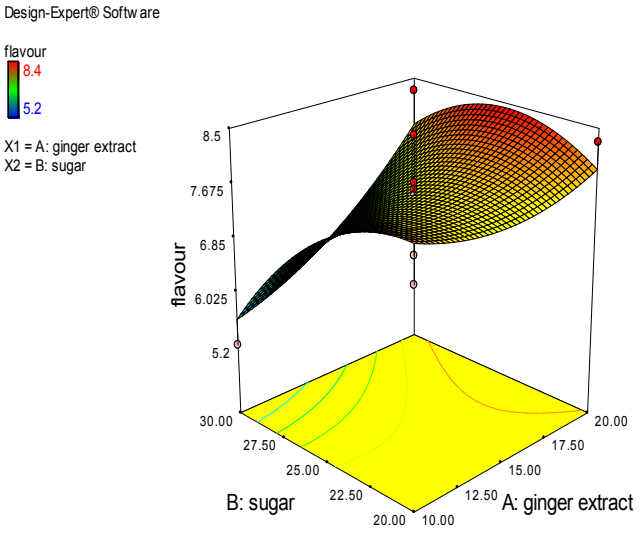


Fig. 2. Response surface and counter plots showing effects of variable on the flavor of value added bread

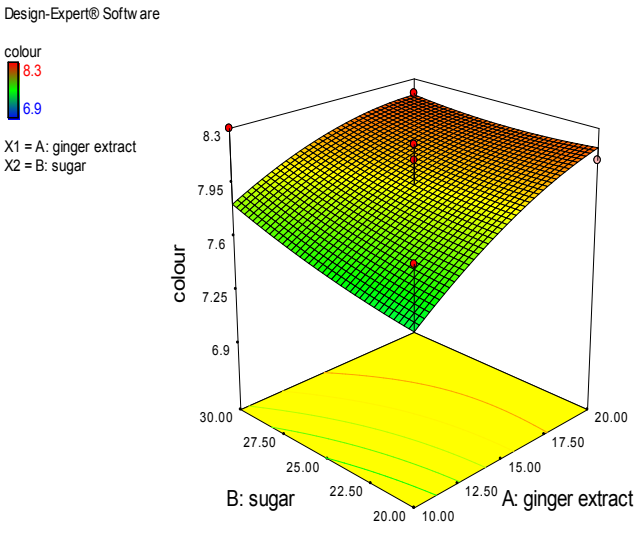


Fig. 3. Response surface and counter plots showing effects of variable on the colour of value added bread

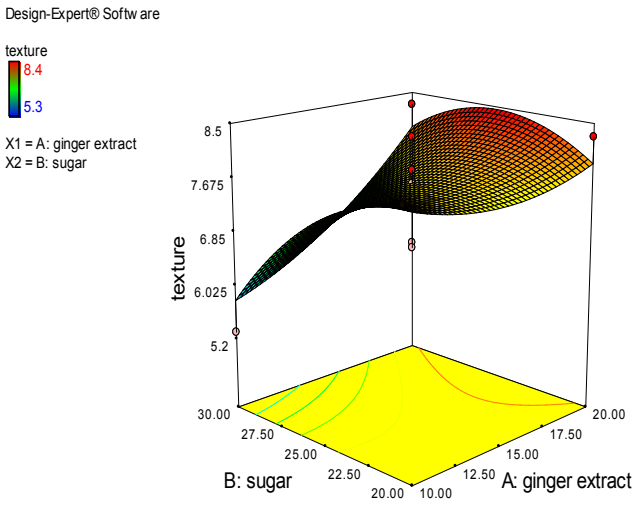


Fig. 4. Response surface and counter plots showing effects of variable on the texture of value added bread

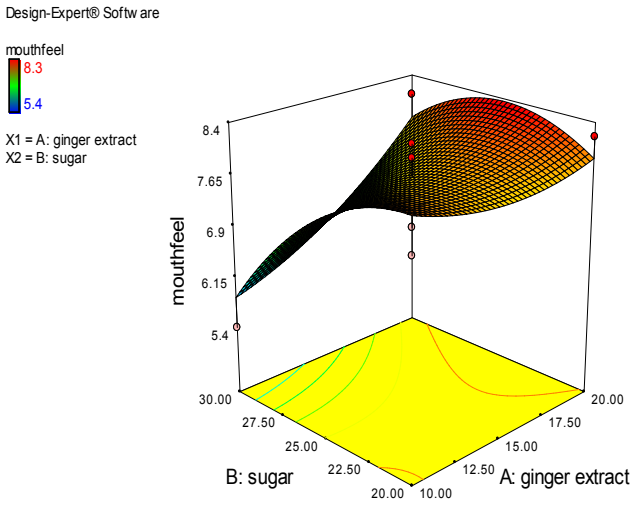


Fig. 5. Response surface and counter plots showing effects of variable on the mouth feel of value added bread

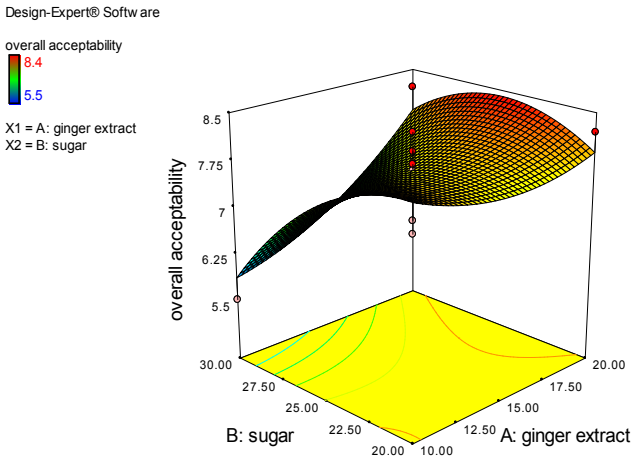
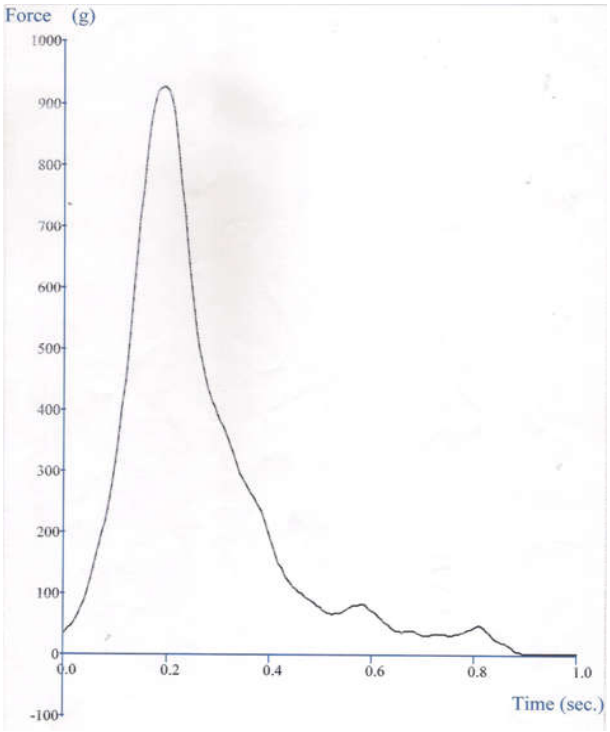
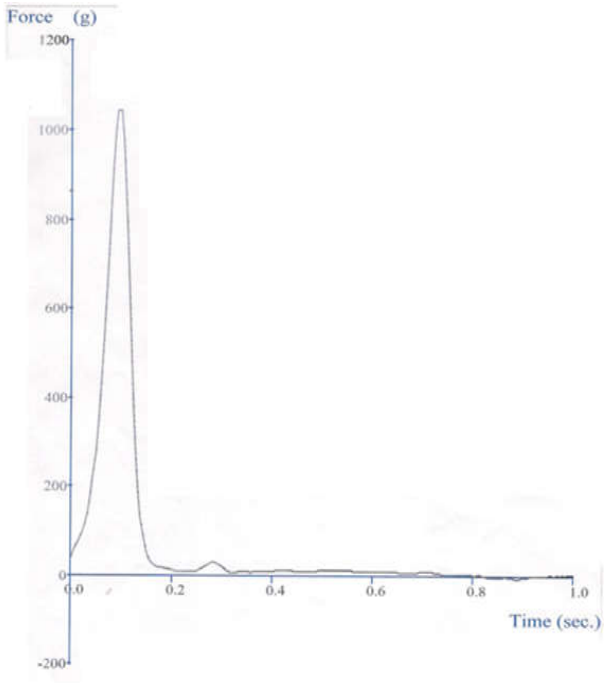


Fig. 6. Response surface and counter plots showing effects of variable on the overall acceptability of value added bread



(a)



(b)

Fig. 6 Effects of variables on the texture of bread: (a) Control bread and (b) Optimized bread

Texture analysis of Optimized Bread

From the results of texture analysis, the hardness of control bread and optimized bread was found to be comparatively near to each other i.e. 915 g and 1015 g respectively (Fig. 6). This result can be correlated with the acceptable sensory scores of control and optimized bread (texture score of 7.9 & 8.3 respectively).

Nutritional Evaluation of Optimized Bread

The nutritional composition of optimized bread regarding moisture, ash, crude fat, crude protein, crude fiber, carbohydrate and mineral contents are presented in (Table 3). The nutritive value of ginger extract incorporated bread was significantly higher than that of control. By incorporation of ginger extract, the ash content was increased from 1.47% to 2.94%. The optimized bread was found to be superior in terms of minerals, calcium, iron and fibre, 23.6, 3.0mg/100g and 2% respectively as compared to control bread (12.1 & 2.5 mg/100g and 0.2%). The moisture content was decreased from 39.5% to 23.5% which may be also helpful for the improvement of shelf-life of optimized bread. Since, the bread was a good source of calcium, iron and fibre (Table 3), hence it can be recommended for consumption for children and old age people.

Evaluation of antioxidant activity

Antioxidant activity in terms of total phenol content and DPPH % radical scavenging activity was found to be 310.54mgGAE / 100g and 93.2% respectively, for ginger extract incorporated optimized bread which was higher than control bread (212.65mg GAE / 100g & 90.1%) (Table 4). The addition of ginger extract, gave an excellent antioxidant effect on the bread compared with control. The same results were obtained in study in which, in bread, addition of purified extracts of marjoram, mint and basil is reported to have an excellent antioxidant effect compared with the effect of BHA (Bassiouny *et al*., 1990). The higher efficiency of the ginger extract could be due to the stability of this natural antioxidant during baking. Results of sensory evaluation reveal that the ginger extract at concentrations of 15% may be used in place of synthetic antioxidants in bread. Addition of natural antioxidants can increase shelf-life of food products containing fats and oils. In addition, natural antioxidants are safe and impart health benefits to the consumer.

Conclusion

Consumption of bakery products in the country is increasing day by day and bread is commonly consumed food like bread, have several attractive features. Nutritionally, bread can be easily fortified with ginger extract to provide a convenient food to supplement the diet's nutrition. The herbal therapeutic benefits of ginger are mainly due to the presence of volatile oils and in the view of health benefit of ginger it may be worthwhile to explore possibility of incorporating ginger extract in wheat flour. Present study was an effort to standardize the level of ginger extract in formulation for the development of value added bread. From the study, it was found that the bread having composition 17.93 g sugar, 15 ml ginger extract per 100 g of bread was found optimum and the said formulation was acceptable and recommended for value added bread. Ginger extract bread provide concentrated form of nutrients along with vitamins and minerals. The nutritive value of ginger extract incorporated bread was significantly higher than that of control. The optimized bread was found to be superior in terms of minerals, calcium and iron as compared to control bread. Since, the bread was a good source of calcium and iron, hence it can be recommended for consumption for children and old age people. The addition of ginger extract, also gave an excellent antioxidant effect on the bread compared with control. Addition of natural antioxidants can increase shelf-life of food products containing fats and oils. In addition, natural antioxidants are safe and impart health benefits to the consumer. It can be recommended as one of the value added products.

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Original article

Wheatgrass incorporation as a viable strategy to enhance nutritional quality of an edible formulation

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Abstract

Wheatgrass is highly valuable due to its medicinal properties. Despite the medicinal properties, wheatgrass could not be part of daily diet, as it is not a regular part of diet so there is requirement to prepare food product supplemented with wheatgrass. In this study, we have prepared edible formulation based on wheatgrass (EFWG). The optimum combination of ingredients for the preparation of wheatgrass incorporated edible formulation was determined using response surface methodology (RSM). RSM is used to analyse the effect of wheatgrass flakes, refined wheat flour, and frying time on sensory and objective (total phenolic and fiber levels) attributes of formulated food product. A central composite rotatable design was used to develop models for the sensory and objective responses. Responses were mostly affected by the changes in wheatgrass flakes level and frying time and to a lesser extent by the refined wheat flour. Responses individual graph plots of different responses were superimposed and regions meeting the maximum sensory score (7.85), total phenolic content (81.85 mg/100 g) and fiber (2.43 g) were identified at 7.00 g wheatgrass flakes, 90.98 g refined wheat flour and 3:50 min. frying time. Optimized formulation was analyzed for its nutritional composition, antinutritional factors and antioxidant properties. The optimized formulation could be recommended to all the age group but especially for children, lactating mothers and geriatric population due to its high calcium, iron and fiber content.

Key words: Wheatgrass, response surface methodology, product formulation, nutritional analysis, antioxidant properties, antinutritional factors

1. Introduction

Wheatgrass is widely used in the Indian traditional system of medicine for various ailments (Rajesh *et al.*, 2011). The young grass of common wheat plant, known as wheatgrass *(Triticum aestivum*), belongs to family poaceae. Wheatgrass is rich in vitamins (A, C, and E), minerals (Ca, Mg, Iron, Zinc), fiber and bioactive compounds (chlorophyllin, quercetin, rutin). Chlorophyll constitutes about 70% of total chemical constituents of wheatgrass (Swati *et al.*, 2010). Several biological properties have been attributed to wheatgrass including blood building activity (Marwaha *et al.*, 2004), anticancer **(**Dey *et al.*, 2006**)**, antiulcerative (Kothari *et al.*, 2008**)**, antidiabetic (Chauhan *et al.*, 2014**)**, antiarthritic (Nenonen *et al.*, 1998), anti-inflammatory and antiageing (Smith *et al.*, 2006). It is believed that pharmacological potential of wheatgrass is due to its high nutrient content and presence of bioactive compounds, which makes it a medicinal plant for the treatment of various diseases and life threatening conditions (Walters *et al.*, 1992).

Ready to eat snacks are an important source of nutrients (energy, protein, iron, calcium and several vitamins). Most ready to eat snacks can easily be enriched and fortified at a low cost with proteins, vitamins and minerals to meet the specific needs of the target groups and vulnerable sections of the population, who are malnourished. Use of fortification and enrichment plays an important role in creating food products (Sharma *et al.*, 1998).

Wheatgrass flakes could be used for preparing value added ready to eat products for organoleptic, economic and nutritional reasons. Value added edible formulation of wheatgrass (EFWG) could be easily fortified with wheatgrass flakes to provide a convenient food to supplement daily nutrition. Since consumption of ready to eat snacks is increasing day-by-day and wheatgrass on the other hand is full of functional ingredients. It may be worthwhile to explore the possibility of incorporating wheatgrass in edible formulations. The present study is an effort to standardize the level of wheatgrass flakes for the development of value added edible formulation of wheatgrass (EFWG).

2. Materials and Methods

2.1 Procurement of the raw material

Wheatgrass seeds for the research were purchased from local market of Allahabad, and grown in controlled conditions at the laboratory of Centre of Food Technology, University of Allahabad, Uttar Pradesh, India. All the other required ingredients like refined wheat flour, common salt, refined oil and spices were purchased from local market of Allahabad. All the chemicals used in analyses were of AR grade.

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2.2 Cultivation of wheatgrass

Wheatgrass was grown in 2 inch deep plastic trays filled with one part organic fertilizer/peat moss and three parts planting soil. Wheat grains were soaked in water for 24 h then rinsed. Wheat was evenly spread over the moist soil. It was covered with a paper towel and placed near a window to ensure proper ventilation for three days. Around the ninth or 10th day, the wheatgrass having grown to 10-12 inches were harvested. At this stage, the wheatgrass is at its nutritional peak. Fresh leaves of the grass were cut and dried in hot air oven (50-55^0 C) for 6 h. Dried samples were stored in airtight containers for further processing.

2.3 Experimental design

Response surface methodology (RSM) was used to determine the experimental design and optimal ingredient level in preparation of EFWG. RSM is an important tool for optimization, which reduces the number of experimental runs needed to provide sufficient information for statistically acceptable results. A three factor central composite design (CCD) was used to design the experiments, comprising of three independent variables including the wheatgrass flakes (6-8%), refined wheat flour (92-95%) and frying time (2-5 min) Table 1. The effects of these variables were seen on the responses variables total phenolic content, fiber and overall acceptability. The experimental sheets of 20 variants with different ratio of independent variables were generated (Table 2). The response variables to be estimated were entered in the sheet. This data were subjected to analysis of variance (ANOVA) one-way analysis and regression coefficients (R^2) to get the optimum response. Coefficient of determination (R^2) values should be close to 1. The predicted R^2 value should be in reasonable agreement with adjusted R^2 (Bunkar *et al.*, 2012). R^2 can be defined as the ratio of explained variation to the total variation, which was a measure of the degree of fit (Chan *et al.*, 2009).

Table 1: Levels of dependent factors to optimized EFWG

Name	Units	(-1) Low level	(+1) High level	(-) Alpha	(+) Alpha
Wheatgrass flakes	(g)	6	8	5.31821	8.68179
Refined wheat flour	(g)	92	95	90.9773	96.0227
Frying time	(Minutes)	2	5	0.977311	6.02269

Table 2: Experimental design generated levels of independent variables in estimated responses

Generated			Estimated		
Wheatgrass flakes (g)	Refined wheat flour (g)	Frying time (minutes)	TPC (mg/100g) Acceptability	Fiber (g)	Overall
6.00	92.00	2.00	80.89	1.65	6.89
8.00	92.00	2.00	81.12	2.26	7.12
6.00	95.00	2.00	81.66	2.15	7.66
8.00	95.00	2.00	80.22	1.96	6.22
6.00	92.00	5.00	79.86	2.42	5.86
8.00	92.00	5.00	81.56	3.15	7.56
6.00	95.00	5.00	80.89	1.85	6.89
8.00	95.00	5.00	81.88	2.66	7.88
5.32	93.50	3.50	80.69	1.89	6.69
8.68	93.50	3.50	80.54	2.42	6.54
7.00	**90.98**	**3.50**	**81.85**	**2.43**	**7.85**
7.00	96.02	3.50	81.56	2.76	7.56
7.00	93.50	0.98	80.89	1.89	6.89
7.00	93.50	6.02	81.65	2.21	7.65
7.00	93.50	3.50	81.12	2.52	7.12
7.00	93.50	3.50	81.12	2.36	7.12
7.00	93.50	3.50	81.12	2.65	7.12
7.00	93.50	3.50	81.45	2.21	7.45
7.00	93.50	3.50	81.25	2.36	7.25
7.00	93.50	3.50	81.52	2.41	7.52

2.4 Formulation of the product

Preparation of the EFWG: Wheatgrass flakes, refined wheat flour and refined oil were mixed in the proportions as obtained in the experimental design to form different formulations. These formulated mixes were further mixed with fixed ingredients, *i.e.*, common salt (2.8 gm), and ajwain (3.5 gm). The dry powder was thoroughly mixed, followed by the addition of refined oil and cold water (25-35 ml), to make a pliable dough. Refined oil was used during the dough preparation to enhance the stability of the product as well as improve the texture of the end product. Small round balls were made from the dough, rolled and flattened into circular shape (20 cm diameter) and cut into desirable shapes. These pieces were fried (according to the combinations) in refined sunflower oil and heated up to 150 ± 5°C to a golden brown colour. The control samples were prepared following the same procedure, without incorporation of wheatgrass flakes. The EFWG were packed in paper/ foil/ polyethylene (PFP) pouches prior to sensory, proximate, antioxidant and antinutritional analysis. The data for formulations along with responses were analyzed using statistical software (Design-Expert 7.0.0) of the best-fit design to obtain the optimized compositions.

2.5 Determination of responses

2.5.1 Total phenolic content (TPC)

Phenolic compound concentration in the EFWG methanolic extract was estimated by a colorimetric assay, based on procedures described by Singleton and Rossi (1965) with some modifications (Singleton and Rossi, 1965). Briefly, 1 ml of sample was mixed with 1 ml of Folin-Ciocalteu's phenol reagent. After 3 min, 1 ml of saturated sodium carbonate solution was added to the mixture and adjusted to 10 ml with distilled water. The reaction tube was kept in the dark for 60 min after which the absorbance was measured at 765 nm (Thermo Scientific, model-Evolution 600). Gallic acid was used to calculate the standard curve (0.01- 0.4 mM). The results are expressed as mg of gallic acid equivalents/g of extract (GAEs).

2.5.2 Estimation of crude fiber

Crude fiber was estimated using acid and alkaline digestion method (Ranganna, 2005). Digestion of 2 g sample (W) was done with 200 ml H_2SO_4 (0.25N) for 30 min. Residue was washed with hot distill water. Then digestion was done with 250 ml of NaOH (0.25N) for 30 min. Again washed with hot distill water. Then residue was washed with 15 ml ethanol. This residue was kept in hot air oven untill constant weight (W1) was obtained. Kept the residue in muffle furnace at 450°C for 4-5 h. Weight (W2) was taken after it get cooled.

$$\text{Crude fiber content (\%)} = (W1-W2)/(W) \times 100$$

2.5.3 Overall acceptability

Sensory evaluation of EFWG (20 combinations) resulting from the experimental design was evaluated in relation to the sensory preference using 9-point hedonic scale with anchor points, 1 (dislike very much) and 9 (like very much). A semi-trained panel of 10 judges evaluated the samples, which were randomly presented for overall acceptability. All panelists were between the ages of 25 to 40 years. The order of presentation of samples was randomized and different 2-digit number codes were used for the sample sets. The coded samples were served at room temperature (25°C) and water was provided for rinsing. The results were presented as mean of 10 evaluations.

2.5.4 Nutritional analysis

The nutritional analysis of the optimum formulation was conducted to evaluate the nutritional adequacy of the formulated food product. Moisture and ash were estimated by using standard (AOAC, 2005) method. Protein was estimated using micro-kjeldhal method using kel plus. Fat was determined by the soxhlet extraction method (Ranganna, 2005). The carbohydrate content was calculated by difference from the levels of nutrients analyzed. Iron and phosphorus were determined according to standard method of AOAC using spectrophotometer while calcium and vitamin C were determined by titrimetric method (Ranganna, 2005). The total caloric value was calculated by using the equation:

$$TCV = [(\text{carbohydrates} + \text{protein})\ 4 + (\text{lipids})\ 9]\ \text{kcal.g}^{-1}$$

2.6 Antinutritional analysis

2.6.1 Tannin

Tannin content in wheatgrass and optimized EFWG was determined by Folin-Denis method (Sadasivum and Manickam, 2005). Color intensity was measured at 700 nm after 30 min of incubation period. Standard graph was prepared using 0-100 µg tannic acid. Tannin content of the samples was calculated as per cent (%) tannic acid from the standard graph.

2.6.2 Phytate

Phytate content was determined by colorimetric method as described by Sadasivam and Manickam (2005). 3% TCA was used for extracting phytate and was precipitated as ferric phytate, which was then converted into ferric hydroxide, and soluble sodium phytate by adding sodium hydroxide in boiling condition. Hot nitric acid was added to it and solution was diluted. Colour of solution was developed using potassium thiocyanate and its intensity was read immediately at 480 nm. The absorbance of iron content so determined was used for calculating phytate phosphorus content assuming a constant 4 Fe: 6 P molecular ratio in the precipitate. Ferric nitrate was used to make standard curve.

2.6.3 Trypsin inhibitor

Trypsin inhibitor (TI) content of sample was determined according to the method of (Kakade *et al.,* 1974) as modified by Rackis *et al.* (1981) using BAPNA (N-a-Benzoyl-DL-Arginine p-nitroanilide) as a substrate.

2.7 Antioxidant analysis

2.7.1 Radical scavenging activity

The free radical scavenging activity of the extract was measured using DPPH (1, 1- diphenyl 2-picryl hydrazyl) method of (Mansouri *et al.,* 2005) with slight modification. 10 mg of grounded optimized product was mixed with 10 ml acidified methanol and heated at 40°C in water bath for 30 min. 100 µl of sample extract thus prepared was kept in a test tube and diluted with 2.9 ml of pure methanol. Sample was mixed with 150 µl of DPPH solution, incubated for 15 min. in dark and absorbance was measured in UV visible spectrophotometer at 515 nm. The % radical scavenging activity was calculated using following formula:

$$\text{Control Absorbance - Sample Absorbance/Control Absorbance} \times 100$$

2.7.2 Ferric reducing antioxidant power (FRAP)

Ferric reducing antioxidant power was determined by the method described by Benzie and Strain (1996). 200 µl of methanolic extract of sample was mixed with 1.3 ml of freshly prepared FRAP reagent and kept for incubation at 37°C for 30 min. Absorbance was measured at 595 nm by using spectrophotometer. The absorbance change in the test mixture was compared with standard mixture of heptahydrate ferrous sulphate (0.1 mM/l - 1.0 mM/l). FRAP values are expressed as mMol of Fe (II) equivalent/ g sample.

2.7.3 Reducing capacity

The reducing power was determined according to the method of Oyaizu (1986). EFWG methanolic extract (2.5 ml) was mixed with 2.5 ml of sodium phosphate buffer (pH 6.6) and 2.5 ml of 1% potassium ferricyanide. The mixture was incubated at 50°C for 20 min. After that, 2.5 ml of 10% trichloroacetic acid (w/v) was added in the mixture and centrifuged at 650 rpm for 10 min. The upper layer (5 ml) was mixed with 5 ml deionised water and 1 ml 0.1% ferric chloride, and the absorbance was measured at 700 nm, higher absorbance indicates higher reducing power. Ascorbic acid was used as standard.

2.7.4 Total flavonoid content

Aluminum chloride colorimetric method was used for flavonoid determination (Bahorun *et al.*, 1996). 1 ml of EFWG methanolic extract was mixed with 1 ml of 2% aluminum chloride. The absorbance of the reaction mixture was measured at 430 nm with a spectrophotometer (Thermo Scientific, model-Evolution 600). A calibration curve was prepared using a standard solution of quercetin (0.05-0.5 mg/ml). Final results are expressed as mg quercetin equivalents/g (QE) of sample.

2.8 Statistical Analysis

The data obtained were analyzed statistically for analysis of variance (ANOVA) using completely randomized design with least significant difference (LSD) at $p < 0.05$ using Design-Expert 7.0.0 statistical software package.

3. Results

In this study, wheatgrass fiber and minerals rich snack food was prepared from natural ingredients to yield products with specific functional properties. The proximate composition of EFWG clearly showed that optimized formulation is rich in calcium (117.33 mg/ 100 g), iron (5.36 mg/100 g), dietary fiber (2.43%) and energy (496.59 kcal), respectively (Table 3). The optimized edible product of wheatgrass (EFWG) was developed using central composite design with minimum possible number of points. The experimental design with different independent variables and respective responses along with the coded variables for the product is given in (Table 2).

3.1 Response surface model

The best formulation was optimized using central composite design (CCD). Three factors were studied to get the optimum values. The experimental sheet of different independent variables; amount of wheatgrass flakes and refined wheat flour with frying time was generated and the responses; estimated total phenolic content, crude fiber and overall acceptability were entered in the sheet (Table 2). The data were analyzed on the basis of ANOVA and regression coefficients. The effects of different independent variables were studied on different responses to obtain an optimum solution. The results of ANOVA and regression coefficient (R^2) for the effect of independent variables on dependent variables, known as responses, were analyzed. For each model, the probability (p value) was less than F value ($p \geq 0.05$), revealing that the terms in each model had a significant effect on the responses-total phenolic content, fiber and overall acceptability. The R^2 values were 0.8408, 0.8079, and 0.8504 for TPC, fiber, and overall acceptability, suggesting a good fit of each model.

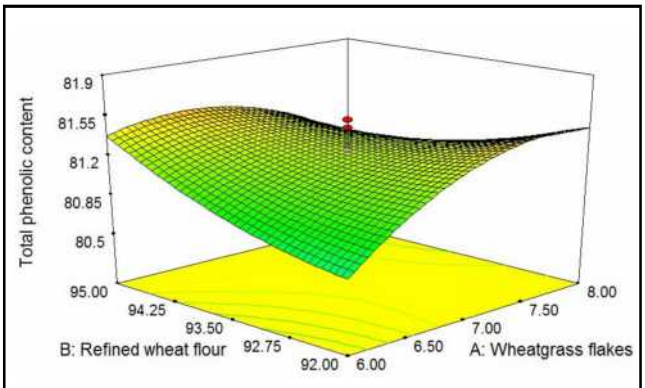
Table 3 : Proximate analysis

Parameters	Control	Optimised EFWG
Moisture (g)	13.00±1.00	11.00±1.00
Protein (g)	6.50±0.78	7.89±0.68
Fat (g)	30.30±2.14	27.85±1.12
Ash (g)	2.00±0.85	2.15±0.65
Carbohydrate (g)	48.2±1.00	49.11±1.15
Energy (kcal/100g)	491.5±0.56	496.59±0.86
Phosphorus (mg)	53.00±1.00	72.33±2.21
Calcium (mg)	100.00±2.00	117.34±2.45
Iron (mg)	2.70±0.20	5.36±1.36
Ascorbic acid (mg)	0.78±0.02	7.00±0.50

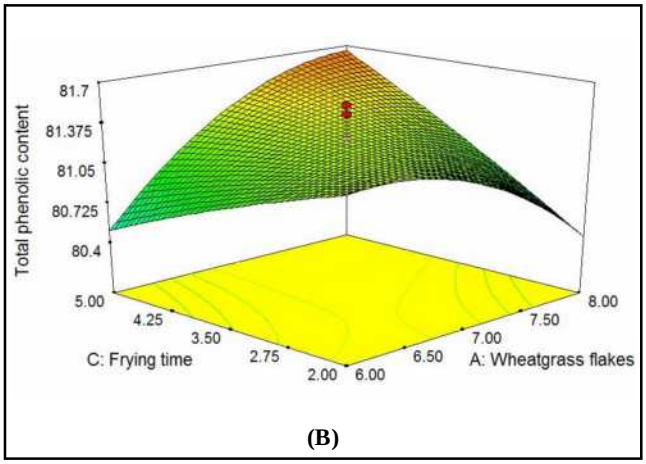
EFWG = Edible formulation of wheatgrass

3.2 Effect of independent variables on total phenolic content

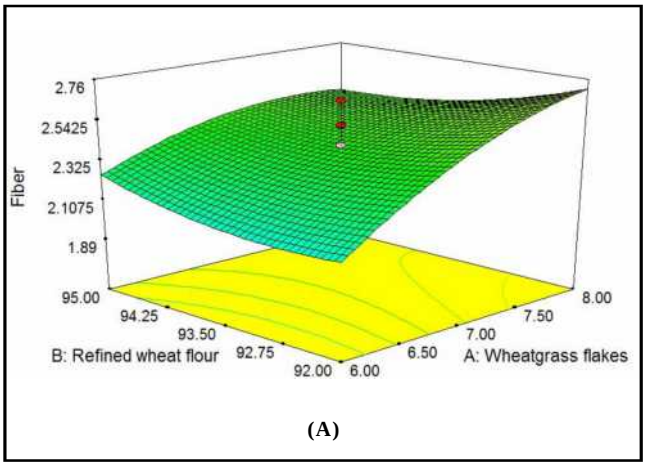
Total phenolic content of EFWG (TPC) is one of the important antioxidant properties of the formulated snacks. In the present study, TPC was found to be in the range of 79.86-81.88 mg/100 g (Table 2). Response surface plot for TPC as function of wheatgrass flakes, refined wheat flour and frying time is given in Figure 1 (a, b, c). Increase in the values of wheatgrass flakes, refined wheat flour and frying time increases the TPC. Regression analysis showed a significant ($p \leq 0.05$) positive effect of wheatgrass flakes, refined wheat flour and frying time on total phenolic content.



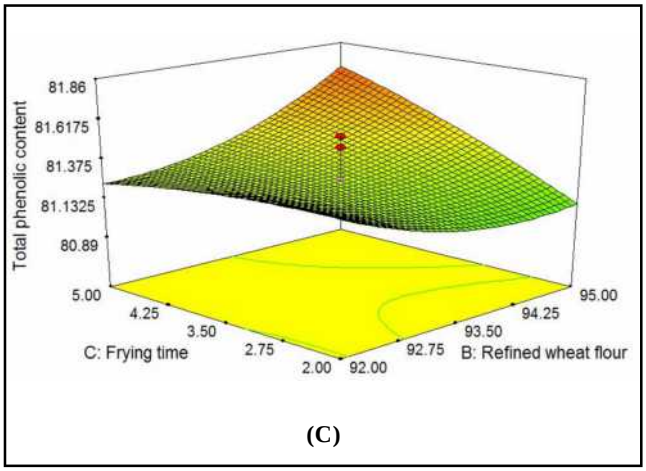
(A)



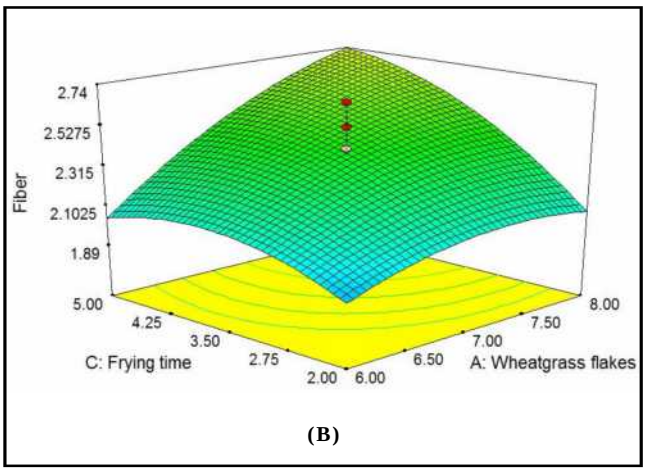
(B)



(A)



(C)



(B)

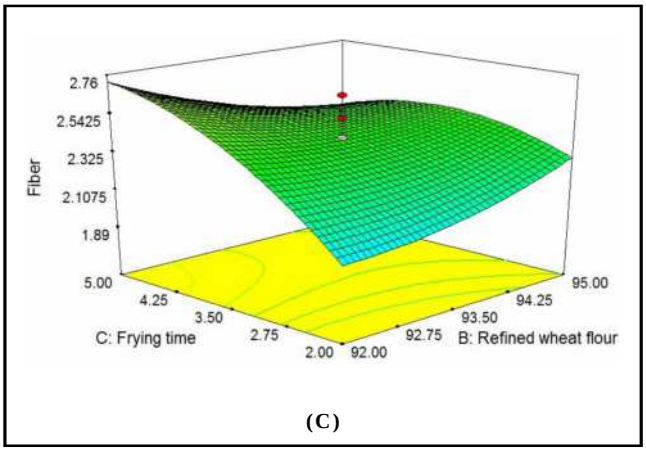
Figure1: Response surface and counter plots showing effects of variable on the total phenolic content of EFWG, (A) wheatgrass flakes vs. refined wheat flour, (B) wheatgrass flakes vs. frying time, (C) refined wheat flour vs. frying time.

3.3 Effect of independent variables on crude fiber content

In this experiment, the fiber content ranged from 1.65 to 3.15 g/ 100 g (Table 2). Regression model fitted well with the experimental results. Fiber content showed a significant ($p \leq 0.05$) positive effect with both the variables; wheatgrass flakes and refined wheat flour however frying time showed ($p \leq 0.05$) negative effect on fiber content. From the response surface plot Figure 2 (a, b, c), it was observed that increase in wheatgrass flakes and refined wheat flour content increases significantly ($p \leq 0.05$) the value of fiber of the optimized formulation.

3.4 Effect of independent variables on overall acceptability

Overall acceptability of EFWG was found to be in the range of 5.86 to 7.85 (Table 2). As per regression coefficient for overall acceptability, it was noticed that overall acceptability of the optimized product (EFWG) showed a positive ($p \leq 0.05$) effect with wheatgrass flakes, refined wheat flour and frying time. Response surface plot Figure 3 (a, b, c) showed that overall acceptability of the optimized product increases with increase in the value of wheatgrass flakes, refined wheat flour and frying time.



(C)

Figure 2: Response surface and counter plots showing effects of variable on the fiber content of EFWG, (A) wheatgrass flakes vs. refined wheat flour, (B) wheatgrass flakes vs. frying time, (C) refined wheat flour vs. frying time.

3.5 Optimization and characterization

The response optimization was achieved as per the desired criteria based on the acceptance of the product. The solutions could be achieved from the software with the maximum desirability as well as the acceptance and the optimum variable levels by being at random starting points and proceeding on the path of the steepest

slope to a maximum. The best among them was taken as the optimum. Wheatgrass flakes 7.00 g, refined wheat flour 90.98 g with 3:50 min frying time achieving the desirability of 1 and OAA of 7.85 on nine point hedonic scale was the optimized ingredient composition with the best fit. The predicted response value of acceptability, TPC and fiber content scores were 7.26, 8.26, 2.41 as against actual values 7.85, 81.85, 2.43, respectively, which were in concurrence with each other.

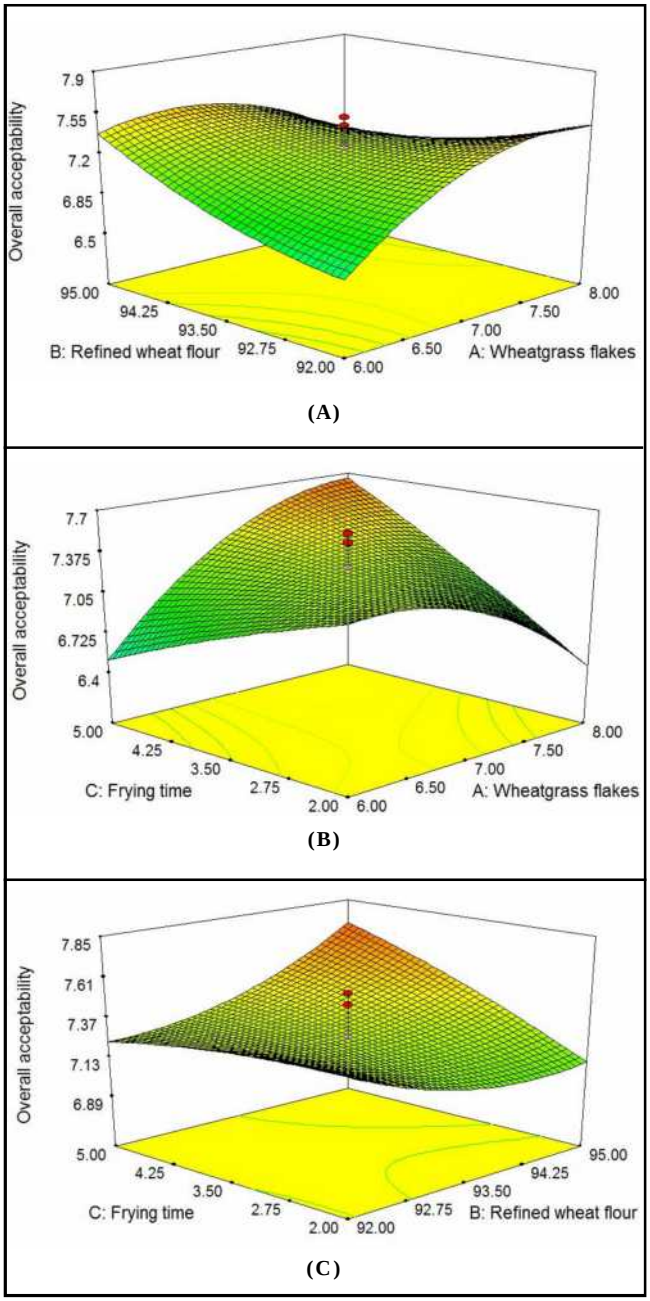


Figure 3: Response surface and counter plots showing effects of variable on the overall acceptability of EFWG, (A) wheatgrass flakes vs. refined wheat flour, (B) wheatgrass flakes vs. frying time, (C) refined wheat flour vs. frying time.

3.6 Proximate composition

The proximate composition of the optimized EFWG is shown in (Table 3). By incorporation of wheatgrass flakes, the protein, ash and carbohydrate was increased from 6.50% to 7.89%, 2% to 2.15%, and 48.2% to 49.11%, respectively. The fat and moisture content was decreased from 13% to 11%, 30.30% to 27.85% with incorporation of wheatgrass flakes. The optimized formulation was found to be superior in terms of 117.34% calcium, 72.33% phosphorus, 5.36% iron, and 7% ascorbic acid as compared to control, 100% calcium, 53% phosphorus, 2.70% iron, and 0.78% ascorbic acid, respectively.

3.7 Antinutritional analysis

The antinutritional factors of optimized product are summarized in (Table 4). Highest tannin, trypsin inhibitor and phytate content was found in optimized EFWG (0.56%, 20%, 38.67%), respectively, and lowest was in case of control (0.43%, 18%, 34.33%), respectively.

Table 4: Antinutritional analysis

Treatments	Tannin (mg/100g)	Phytate (%)	Trypsin inhibitor (%)
Control	0.43±0.11	34.33±1.52	18.00±1.25
Optimised EFWG	0.56±0.21	38.67±1.52	20.00±1.41

EFWG = edible formulation of wheatgrass

3.8 Antioxidant analysis

Antioxidant potential of optimized formulation EFWG was shown in (Table 5). Wheatgrass flakes supplemented optimized formulation contained higher antioxidant potential including 98.67 mgQE/g total flavonoid content, 78.33% DPPH radical scavenging activity, 0.42 mmol Fe(II)Eq/g FRAP value, 0.41 % reducing capacity, and 46.67 umolAAE/g metal chelating activity than control (40 mg QE/g, total flavonoid content, 47.67% DPPH radical scavenging activity, 0.21 mmol Fe(II)Eq/g FRAP value, 0.26 % reducing capacity, 17 umolAAE/g Metal chelating activity, respectively.

Table 5 : Antioxidant analysis

Treatment	TFC (mg QE/g)	DPPH (%)	FRAP (mmolFe(II)Eq/g)	Metal chelating (µmol/AAE/g)	Reducing powder (%)
Control	40.00±2.54	47.67±2.5	0.21±0.23	17.00±0.77	0.26±0.23
Optimised EFWG	98.67±7.5	78.33±1.5	0.42±0.15	46.67±0.88	0.41±0.06

4. Discussion

In this study, response surface methodology was used for the optimization of independent variables, *i.e.*, amount of wheatgrass flakes, amount of refined wheat flour and frying time and their effect on responses, *i.e.*, total phenolic content, fiber and overall acceptability. It reveals that the terms in each model had a significant effect on the responses-total phenolic content, fiber and overall acceptability, suggesting a good fit of each model. Jackson *et al.* (1996) used RSM to predict that maximum crispness of banana chips could be produced by blanching at 69°C and 22 min, while Shyu and Hwang (2001) optimized the conditions for vacuum frying

of apple chips at temperature of 100-110°C for 20-25 min. and immersing in fructose solution of 30-40%.

The increased effect of independent variables on TPC may be due to the higher antioxidant content of wheatgrass flakes and incorporation of refined wheat flour may also have increased TPC content. In some conditions, heat-processing treatments (frying, roasting) may also be helpful for increasing antioxidant content. Heat treatments (frying and roasting) leads to chemical oxidation of phenol and non-enzymatic browning reaction associated with strong antioxidant potential (Manzocco *et al.,* 2000).

Formulated product has increased value of fiber content with incorporation of wheatgrass flakes and refined wheat flour could be due to the high fiber content of wheatgrass. Wheatgrass powder was found to be a rich source of dietary fiber. Mogra and Chouhan (2014) reported higher amounts of dietary fiber content (23.26%) in wheatgrass powder.

For the evaluation of sensory attribute of formulated product, overall acceptability was considered as response variable. In this study the hedonic ratings of sensory attribute, *i.e.*, overall acceptability was observed 7.85 (like moderately) by the panelists (Table 2). Overall acceptability of the optimized product was found increase with increase in the amount of wheatgrass flakes, refined wheat flour and frying time. Crispiness and other sensory attributes were increased with the increased incorporation of wheatgrass flakes in the formulated product.

The nutritive value of wheatgrass flakes supplemented formulation was found higher than that of control product. It is clear that supplementation of the basic formula with the wheatgrass flakes resulted in higher dietary fiber, and mineral matter content. This fulfills approximately one third nutritional requirement of school going children (Table 3). The fiber content was relatively high in this product, which indicates that incorporation of natural plant fibers, and their minerals in food products thus increasing the mineral and fiber consumption in daily diet.

It must be noted that antinutritional factors (tannin, trypsin inhibitor and phytate content) of EFWG was found higher than control product. The results are closer to that of Udensi *et al.* (2007). Studies suggest that antinutritional factors can be reduced by various food processing techniques.

Incorporation of wheatgrass flakes, gave an excellent antioxidant effect on the EFWG as compared with control. Addition of wheat grass enhanced the antioxidant effect of the optimized formulated product. The higher efficiency of the wheatgrass flakes could be due to the persistence of this natural antioxidant during processing. In addition, natural antioxidants are safe and impart health benefits to the consumer.

5. Conclusion

The EFWG formulation can serve as a good source of dietary fiber, minerals and is a novel approach for increasing the mineral and fiber consumption in daily diet. Hence, it can be recommended for consumption by children and old age people for partial fulfillment of nutritional requirements. Wheatgrass may be good source of natural antioxidants and has the potential to enhance the health benefits to the consumer.

Conflict of interest

We declare that we have no conflict of interest.

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DEVELOPMENT AND CHARACTERIZATION OF ANTIOXIDANT RICH WHEATGRASS CUPCAKE

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ABSTRACT

The optimum formulation for production of an Indian traditional baked wheatgrass cupcake was determined using response surface methodology. Effects of amount of ingredients such as wheatgrass powder (5-15%), and baking time (15–35 min) on the antioxidant potential (total phenolic content, total flavonoid content, % DPPH radical scavenging activity and vitamin C), mineral (Iron) and sensory attributes (overall acceptability) of cakes were investigated. Significant regression models which explained the effects of different percentages of wheatgrass powder, and baking time on all response variables were determined. The coefficients of determination, R^2 of all the response variables were higher than 0.83. Based on the response surface and superimposed plots; the basic formulation for production of baked wheatgrass cupcake with desired sensory quality was obtained by incorporating with 5% of wheatgrass powder, and 35 minutes of baking time. Optimized formulation was analyzed for its nutritional composition, antioxidant properties and anti-nutritional factors. The optimized formulation could be recommended to all the age group but especially for children, lactating mothers and geriatric population due to its high antioxidants, iron, calcium, and fiber content.

1. Introduction

Wheatgrass is an integral part of traditional Indian medicinal system. The young grass of common wheat plant is known as wheat grass *(Triticum aestivum*) belongs to family poaceae. Wheatgrass is rich source of antioxidants, vitamins (A, C, E known as an antioxidant), minerals (ca, mg, iron, zinc etc.), fiber and bioactive compounds (chlorophyllin, qurecitin, apegenine and rutin). The foremost constituent of Wheatgrass is chlorophyll. Chlorophyll constitutes about 70% of total chemical constituents of Wheatgrass (Swati et al. 2010). Chlorophyll which is presence in wheatgrass has almost chemically comparable to hemoglobin. It has been various pharmacological potential, to have blood building activity (Marwaha et al., 2008), anti cancer activity (Dey et al.,2006), anti ulcer activity (Ben-Arye 2002), anti diabetic potential (Chauhan et al., 2014), anti arthritic potential, anti inflammatory and anti aging potential (Smith et al., 2000). It is believed that pharmacological potential of wheatgrass is due to its high nutrient content and presence of bioactive compounds, which makes it a medicinal plant for the treatment of various diseases and life threatening conditions (Walters et al., 1992). With such enormous health benefits, the present study was conducted to optimize the formulation of wheatgrass cupcake of rewarding sensory attribute, nutritional properties and antioxidant content of the developed wheatgrass cupcake.

Cupcake is known to be one of the most expedient and accepted bakery product in the world (Udeme et al., 2014) . During the past,

many experiments were conducted to improve the nutritional value of cupcake like fiber rich, sugar free, antioxidant rich cupcake and fat free cupcake. Now days the renewed costumer's interest in the consumption of nutritious healthy and natural food products that leads to various health benefits.

Therefore, the concentrations used in making the cupcake with incorporation of wheat grass powder has been an important factor in developing a new product with less cost and other more benefits such as improving the aesthetic value, nutritional density, antioxidant and fibre content of cupcake. In this context, the main idea of this work was to develop sustaining and functional food products (Tripathi et al. 2017). The aims of this study were i) optimization of the developed food product. ii) To evaluate the proximate composition, iii) antioxidant potential iv) Anti nutritional factors of the developed product.

2. Materials and methods

2.1. Procurement of the raw material

Wheatgrass seeds for the research were purchased from local market of Allahabad, India and grown in controlled conditions at the laboratory of Centre of Food Technology, University of Allahabad, India. All the other required ingredients like refined wheat flour, sugar, milk, butter, baking powder, and coco powder were purchased from local market of Allahabad. All the chemicals used in analyses were of AR grade.

2.2. Cultivation of wheatgrass

For growing wheatgrass, Superior fine quality wheat was procured from local market of Allahabad, and cleaned properly. The wheat grains were soaked in cold water for 12 hours. After 12 hours of soaking the water was strained and the soaked grains were tied in wet woven cotton cloth and hung for a period of 12 hours. After 12 hours of germination, the germinated wheat was sowed in a shady place. Since wheat can grow in all temperatures, shady place is preferred to avoid excess nutrient loss due to exposure to direct sunlight. The sowed seeds started to grow and on the seventh day, the grass reached the length of 15 to 18cm which was then harvested.

2.3. Preparation of wheatgrass powder

Fresh and whole wheatgrass leaf was washed with water, and dried in a cabinet tray dryer (Chemida, Mumbai, India) at 55 ± 2°C for 8 h. The dried material was ground to powder using a high speed mixer (Sumeet Domestic Plus, M/s. Sumeet, Nashik, India), passed through BS 72 (220 μm) mesh and dehydrated whole wheatgrass powder was obtained. The powder was packed in metallized polyester polyethylene (MPE) laminate pouches (12 μm metallized polyester, 7.5 μm polyethylene) laminated pouches of size 14×12 cm^2 were used for packing and stored at 4°C for further chemical analysis and application studies.

2.4. Experimental design

Response Surface Methodology (RSM) was used to determine the experimental design and optimal ingredient level in preparation of wheatgrass cupcake. RSM is an important tool for optimization, which reduces the number of experimental runs needed to provide sufficient information for statistically acceptable results. A three factor central composite design CCD was used to design the experiments comprising of two independent variables including the wheatgrass powder (5-15 g), and baking time (15-35 minutes) Table 1. The effects of these variables were seen on the responses variables total phenolic content, total flavonoid content, % DPPH scavenging activity, vitamin C content, Iron content and overall acceptability. The experimental sheets of 13 variants with different ratio of independent variables were generated. The response variables to be estimated were entered in the sheet. This data was subjected to analysis of variance (ANOVA) one-way analysis and regression coefficients (R^2) to get the optimum response. Coefficient of determination (R^2) values should be close to 1. The predicted R^2 value should be in reasonable agreement with adjusted R^2 (Bunkar et al.,2012). R^2 can be defined as the ratio of explained

variation to the total variation, which was a measure of the degree of fit (Chan et al.,2009).

2.5. Formulation of the product

2.5.1. Preparation of the wheatgrass cupcake

A cake batter recipe containing 100% refined wheat flour,100% sugar, 25% shortening (butter), 9% cocoa powder, 3% salt and 5% baking powder(all percentages are given on a flour weight basis)was used in the experiments. Amount of water added to the batter was 27% of the overall formulation. Wheatgrass powder (5-15%) was mixed in the proportions as obtained in the experimental design to form different formulations. A cake batter containing no wheatgrass powder was used as control (Deora et al., 2014). During preparation of the cake, first, dry ingredients (refined wheat flour, baking powder, salt and wheatgrass powder) were mixed thoroughly. In a separate cup, sugar and butter were mixed, and then melted shortening was added and mixed for 1min at 85rpm by using a mixer (KitchenAid,5K45SS,St.Joseph,MI,USA).
Then, dry ingredient mix and water were added simultaneously to this mixture and mixed first for 2 min at 85 rpm, then for 1min at 140 rpm and finally for 2min at 85rpm.. In cupcake molds, cake samples of 100 g each were baked in microwave oven at 180±5 ºC for 30 minutes (Jerome et al., 2019). Wheatgrass cupcake was packed in paper/ foil/ polyethylene (PFP) pouches prior to sensory, proximate, antioxidant and anti-nutritional analysis. The data for formulations along with responses were analyzed using statistical software (Design-Expert 7.0.0) of the best-fit design to obtain the optimized compositions.

2.6. Sensory Evaluation

The sensory evaluation of the wheatgrass cupcake (13 formulated combinations) was performed by 20 semi-trained panelists from the Department of Food Science and Technology, University of Allahabad, India. The sensory evaluation was conducted using the seven-point hedonic scale as described by Watts, Ylimaki, and Jeffery (1989). The food samples were randomized, coded with three-digit random numbers and each sample was presented with different number. The randomized order of the sample was presented once at a time to each panelist. Panelists were asked to evaluate the coded samples for each sensorial parameter including color, aroma, texture, flavor, and overall acceptability based on their degree of liking (1 = dislike very much; 2 = dislike moderately; 3 = dislike slightly; 4 = neither like nor dislike; 5 = like slightly; 6 = like moderately; 7 = like very much).

2.7. Nutritional analysis

The moisture content of the wheatgrass cupcake was determined by drying at 105 °C until a constant weight was attained as per (AOAC 2005). The micro Kjeldhal method was employed to determine the total nitrogen and the crude protein content (Nx6.25) (AOAC 2005). Crude lipid was estimated by extraction with petroleum ether (60–80 °C), with a soxhlet apparatus and ash contents were determined as per (AOAC 2005). Dietary fiber was estimated using acid and alkaline digestion method. Ash and carbohydrate contents were determined by (AOAC 2005) method. Vitamin C was determined by titrimetric method (Ranganna 2005). Calcium content was estimated by precipitating it as calcium oxalate and titrating with standard potassium permanganate solution; iron content was estimated using colourimetric method using UV–Visible spectrophotometer (Shimadzu, UV-160A model) at 480 nm (AOAC, 2005). Phosphorous content was analyzed by developing colour using ammonium molybdate and 2, amino −6, naphthol sulphonic acid. The blue colour developed was read at 650 nm in UV–Visible spectrophotometer and expressed as phosphorus mg/100 g. The percent carbohydrate content and the energy value were calculated by difference using the following equations:

$$\%\ Carbohydrate = [100 - (Moisture + Total\ ash + Protein + Fibre + Fat)] \quad \text{Eq.1}$$

Energy (kcal/100g) = 4 (% Protein + % Carbohydrate) + 9(% Fat) Eq.2

2.8. Antioxidant analysis

2.8.1. Total Phenolic Content (TPC)

Phenolic compound concentration in the extract was estimated by a colorimetric assay, based on procedures described by Singleton and Rossi with some modifications (Singleton and Rossi 1965). Briefly, 1 ml of sample was mixed with 1 ml of Folin-Ciocalteu's phenol reagent. After 3 min, 1 ml of saturated sodium carbonate solution was added to the mixture and adjusted to 10 ml with distilled water. The reaction tube was kept in the dark for 60 minutes after which the absorbance was measured at 765 nm (Thermo Scientific, model-Evolution 600). Gallic acid was used to calculate the standard curve (0.01– 0.4 mM). The results are expressed as mg of gallic acid equivalents/g of extract (GAEs).

2.8.2. Total flavonoid content (TFC)

Aluminum chloride colorimetric method was used for flavonoid determination (Bahorun et al. 1996). 1 ml of sample methanolic extract was mixed with 1 ml of 2% aluminum chloride. The absorbance of the reaction mixture was measured at 430 nm with a spectrophotometer (Thermo Scientific, model-Evolution 600). A calibration curve was prepared using a standard solution of quercetin (0.05- 0.5 mg/ml). Final results are expressed as mg quercetin equivalents/g (QE) of sample.

2.8.3. Radical scavenging activity

The DPPH radical was used to measure the free radical scavenging activity of extracts by the method of Blois et al 1956. Sample extracts were taken and 3 mL of a 0.1 mmol/L methanolic solution of DPPH was added to the aliquots of sample extracts of product and standards. DPPH solution (3 mL) along with methanol (100µL) was used as a negative control. All the reaction mixtures were incubated for 20 min in dark. DPPH radical inhibition by the samples was measured at 517 nm against the blank (methanol). The inhibition percentage for scavenging DPPH radical was calculated according to the equation:

% decolorization = Control Absorbance - Sample Absorbance/Control Absorbance × 100

2.8.4. Ferric Reducing Antioxidant Power (FRAP)

The ferric reducing ability of the extract was estimated by the method of Pulido et al., 2000. The FRAP reagent was prepared by mixing 2.5 mL of 10 mmol/L TPTZ in 40 mmol/L HCl, 2.5 mL of 20 mmol/L $FeCl_3 \cdot 6H_2O$ and 25 mL of 0.3 mol/L acetate buffer (pH 3.6). 900 µL of FRAP reagent was mixed with 10 µL of aliquot of sample extracts and incubated at 37ºC. After incubation, ferric reducing ability of sample extracts was measured at 595 nm. The results were expressed as µmol/L Fe (II) equivalents/mg extract.

2.8.5. Reducing capacity

The reducing power ability of the extract was evaluated by the method described of Oyaizu et al., 1986. The reaction mixture contained 1.0 mL of product extract (2–10 mg/mL), 2.5 mL of 1% potassium ferricyanide and 2.5 mLof 0.2 mol/L sodium phosphate buffer. The mixture was incubated at 50°C for 20 min and the reaction was terminated by the addition of 2.5 mL of 10% trichloroacetic acid, followed by centrifugation at 3000 r/min for 10 min. 2.5 mL of the upper layer was mixed with 2.5 mL of deionized water and 0.5 mL of 0.1% ferric chloride. The absorbance was measured at 700 nm against blank that contained distilled water and phosphate buffer. Increase in absorbance indicates increased reducing power of the sample. Ascorbic acid was used as standard.

2.8.6. Metal ion chelating activity

The chelating activity of the sample was determined by the method of Dinis et al., 1994. 500 µL of samples were added to 100 µL solution of 2 mmol/L $FeCl_2$. The reaction was initiated by the addition of 400 µL of 5 mmol/L ferrozine and incubated at room temperature for 10 min. Absorbance of the samples was then measured spectrophotometrically at 562 nm against the blank (deionized water). A lower

absorbance of the reaction mixture indicated a higher Fe^{2}+ chelating ability. The control contained all the reagents except sample. Gallic acid and ascorbic acid was used as standard.

2.9. Anti-nutritional analysis

2.9.1. Tannin

Tannin content in optimized product was determined by Folin-Denis method as described by Sadasivum and Manickam (2005). Color intensity was measured at 700 nm after 30 minutes of incubation period. Standard graph was prepared using 0-100 µg tannic acid. Tannin content of the samples was calculated as per cent (%) tannic acid from the standard graph.

2.9.2. Phytate

Phytate content is determined by colorimetric method as described by Sadasivam and Manickam (2005). 3% TCA was used for extracting phytate and was precipitated as ferric phytate, which was then converted into ferric hydroxide, and soluble sodium phytate by adding sodium hydroxide in boiling condition. Hot nitric acid was added to it and solution was diluted. Colour of solution was developed using potassium thiocyanate and its intensity was read immediately at 480nm.The absorbance of iron content so determined was used for calculating phytate phosphorus content assuming a constant 4 Fe: 6 P molecular ratio in the precipitate. Ferric Nitrate was used to make standard curve.

2.9.3. Trypsin inhibitor

Trypsin inhibitor (TI) activity of sample was determined according to the method of Kakade et al.,1974, as modified by (Rackis et al. 1981) using BAPNA (N-a-Benzoyl-DL-Arginine p-nitroanilide) as a substrate.

2.10. Statistical Analysis

The data obtained was analyzed statistically for analysis of variance (ANOVA) using completely randomized design with least significant difference (LSD) at $P < 0.05$ using Design Expert 7.1 statistical software package.

3.Results and discussions

In this study, antioxidant rich wheatgrass cup cake was prepared from natural ingredients to yield products with specific functional properties. The proximate composition of wheatgrass cupcake clearly showed that optimized formulation is rich in calcium (273mg/100g), iron (9.25mg/100g), dietary fiber (12.43%) and energy (433.3 kcal), which fulfills approximately one third nutritional requirement of school going children. The optimized edible product of wheatgrass cupcake was developed using Central Composite Design with minimum possible number of points (Table 1).

The experimental design with different independent variables and respective responses along with the coded variables for the product is given in (Table 2).

Table 1. Levels of dependent vaibles for optimized wheatgrass cupcake.

Variables	Units	(-1) Low level	(+) High level	(-) Alpha	(+) Alpha
Wheatgrass powder	(g)	5	15	2.92893	17.0711
Baking time	(Minutes)	15	35	10.8579	39.1421

Table 2. Experimental data for antioxidant rich wheatgrass cupcake response variables such as wheatgrass powder (g) and baking time (min).

Process variables (coded terms)		Responses					
wheatgrass powder (g)	Baking time (minutes)	TPC (mg/100g)	TFC (mg/100g)	DPPH (%)	Vitamin C (mg/100g)	Iron (mg/100g)	Overall acceptability
5.00	15.00	10.65	0.46	64.58	9.58	8.56	8.24
10.00	25.00	13.25	0.58	80.25	10.37	11.25	7.46
10.00	25.00	14.45	0.61	81.56	11.35	10.65	7.46

10.00	25.00	14.56	0.62	74.58	12.15	11.65	6.49
10.00	25.00	15.45	0.65	80.56	11.65	10.54	7.86
10.00	39.14	16.38	0.64	81.68	10.26	12.56	6.62
10.00	10.86	17.59	0.62	78.68	11.35	11.68	7.46
5.00	35.00	11.56	0.59	71.68	8.56	6.26	6.86
15.00	15.00	13.56	0.64	80.58	11.59	10.47	4.28
15.00	35.00	14.56	0.67	78.68	12.54	16.48	6.46
2.93	25.00	7.54	0.48	58.56	6.48	4.46	6.57
17.07	25.00	14.65	0.78	78.59	13.48	16.46	4.36
10.00	25.00	13.54	0.66	74.59	11.64	15.84	6.65

Model fitting from RSM

The effects of wheatgrass powder and baking time on total phenolic content (TPC), total flavonoid content (TFC), DPPH, iron, and overall acceptability of baked wheatgrass cup cake are shown in Table 2.

The independent and dependent variables were fitted to the second-order model equation and examined for the goodness of fit. The analyses of variance were performed to determine the lack of fit and the significance of the linear, quadratic and interaction effects of the independent variables on the dependent variables (Table 3).

The lack of fit test is a measure of the failure of a model to represent data in the experimental domain at which points were not included in the regression Varnalis et al, 2004.

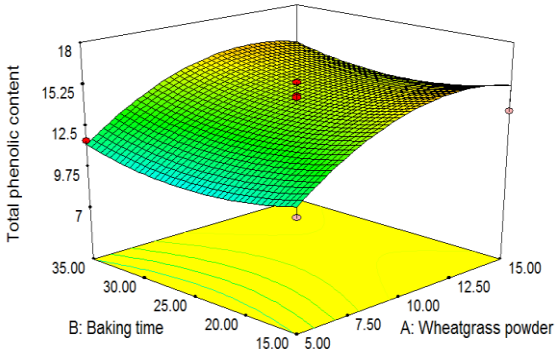

Figure 1. Response surface plot of the effects of wheatgrass powder (g) and baking time (min.) on total phenolic content (TPC) of wheatgrass cupcake.

Table 3. Estimated coefficient for the different response variables

Variables	D.f	Estimated Coefficients					
		TPC	TFC	DPPH	Vitamin C	Iron	O.A.
Model	5	14.25	0.62	78.31	11.43	11.99	7.18
A	1	2.00	0.086	6.42	1.99	3.64	-0.94
B	1	0.025	0.024	1.18	-0.20	0.62	-0.048
AB	1	0.023	-0.025	-2.25	0.49	2.08	0.89
A^2	1	-1.94	-0.0070	-4.99	-0.68	-0.97	-0.81
B^2	1	1.00	-0.0070	0.81	-0.27	-0.14	-0.020
R^2	0.8617	0.8370	0.9146	0.9012	0.9012	0.8442	0.8732
Adj R^2	0.7630	0.7206	0.8535	0.8306	0.8306	0.7329	0.7826
CV%	9.22	6.97	3.57	6.96	6.96	16.5	8.30

TPC=Total phenolic content; TFC= Total flavonoid content; O.A=Overall acceptability; R^2=Coefficient of multiple determinations; CV= coefficient of variance.

Coefficient of determination or R^2 is the proportion of variation in the response attributed to the model rather than to random error and was suggested that for good fit model, R^2 should be at least 80%.

The results showed that the models for all the response variables were highly adequate because they have satisfactory levels of R^2 of more than 80% and that there is no significant lack of fit in all the response variables indicating a high proportion of variability as explained by the data. Therefore, the response surface models developed were adequate.

Effect of amount of wheatgrass powder and baking time

The effect of different amount of wheatgrass powder and baking time on the instrumental data (TPC, TFC, DPPH, ascorbic acid and Iron content) and the sensory attributes (overall acceptability) of baked wheatgrass cupcake are reported (Table 3) by the coefficient of the second-order polynomials (Rifna et al., 2019) . To aid visualization, the response surfaces for these response variables are shown in Figs. 1–6.

Effect on the Total phenolic content (TPC)

Total phenolic content (TPC) is one of the important antioxidant properties of the formulated product. In the present study, It can be observed (Fig.1) that the total phenolic content (TPC) of the baked wheatgrass cupcake depended on the amount of the wheatgrass powder added, as its linear, quadratic and interaction effects were positive at $p \leq 0.05$. Thus, an increase in the amount of wheatgrass powder might probably lead to an increase in total phenolic content of product. This may be due to the higher antioxidant content of wheatgrass powder. Similarly, the effect of baking time showed positive linear, interaction and quadratic effects $p \leq 0.05$) on the phenolic content of baked wheatgrass cupcake. Because in some conditions, heat-processing treatments (baking, roasting) may also be helpful for increasing antioxidant content. Heat treatments (baking and roasting) leads to chemical oxidation of phenol and non-enzymatic browning reaction associated with strong antioxidant potential (Manzocco et al.,2000).

Effect on total flavonoid content (TFC)

Total flavonoid content of backed wheatgrass cupcake was shown in Table 3, and Fig. 2, it is clear that the scores for total flavonoid content were affected by the backing time and amount of wheatgrass powder added. Table 3 showed that total flavonoid content was affected by the amount of wheatgrass powder used, with positive linear and negative quadratic and inaction effects at $p \leq 0.05$. The total phenolic content was high initially and it decreases as the amount of wheatgrass was increased gradually. However, the interaction and quadratic effects of baking time on total flavonoid content were negative at $p \leq 0.05$ and the effect was linear, owing to a positive a $p \leq 0.05$ (Table 3). As the baking time was increased, it had changed the product total flavonoid content.

Hence, a higher amount of wheatgrass powder and moderate level of baking time might increase the total flavonoid content of baked wheatgrass cupcake.

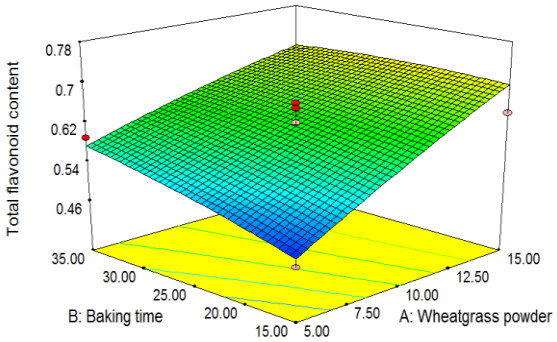

Figure 2. Response surface plot of the effects of wheatgrass powder (g) and baking time (min.) on total flavonoid content (TFC) of wheatgrass

Effect on DPPH radical scavenging activity

Replacement of wheatgrass powder had a positive effect on the DPPH radical scavenging activity indices at positive linear and negative quadratic terms, showing significant levels at $p<0.001$ and $p<0.001$, respectively (Table 3). However It can be observed that the positive effect of baking time on the DPPH radical scavenging activity at linear ($p<0.001$) and quadratic ($p<0.05$) term (Table 3). Thus increasing the replacement level of wheatgrass powder and baking time would increase the DPPH radical scavenging activity indices to positive values. Result also showed that the interaction effect on DPPH ($p \leq 0.05$) was negative meaning that the DPPH was dependent on both of these variables. DPPH content was increased when increase in the amount of wheatgrass powder added and with prolonged baking time (Fig. 3).

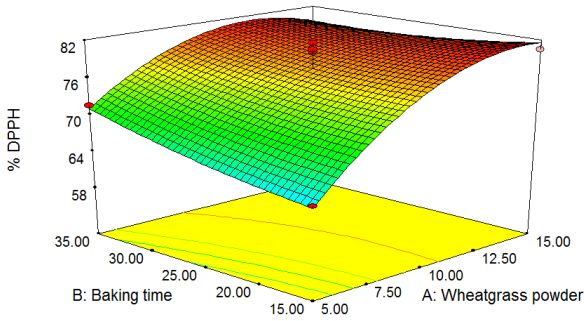

Figure 3. Response surface plot of the effects of wheatgrass powder (g) and baking time (min.) on total % DPPH (TPC) of wheatgrass cupcake.

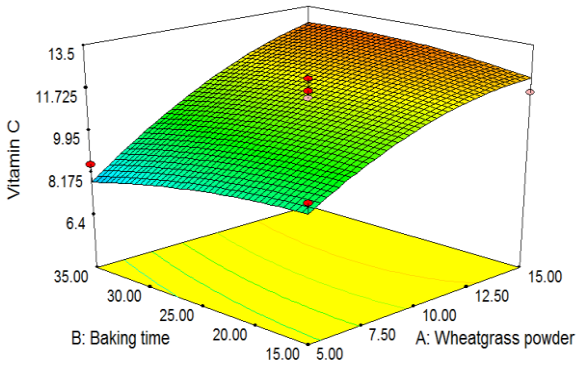

Figure 4. Response surface plot of the effects of wheatgrass powder (g) and baking time (min.) on ascorbic acid of wheatgrass cupcake.

Effect on ascorbic acid content

Baking time had a negative effect on the Ascorbic acid content at linear and quadratic terms, showing significant levels of $p \leq 0.05$ (Table 4).because ascorbic acid is not heat sable and its destroy when temperature is high, a positive linear effect $p \leq 0.05$ of the amount of wheatgrass powder on the ascorbic acid content was found. This indicates that the presence of wheatgrass powder could enhance the ascorbic acid content of the baked wheatgrass cup cake (Fig. 4). The highest amount of ascorbic acid content for baked wheatgrass cupcake obtained when the amount of wheatgrass powder added was increased and baking time deceased.

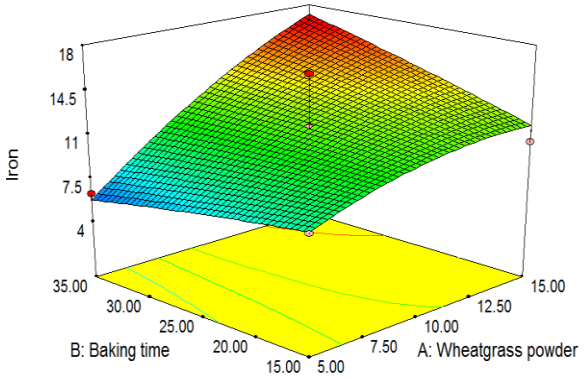

Figure 5. Response surface plot of the effects of wheatgrass powder (g) and baking time (min.) on iron content of wheatgrass cupcake.

Table 4. Analysis of variance for the response variables

Variables	D.f	F-Values					
		TPC	TFC	DPPH	Vitamin C	Iron	O.A.
Model	5	8.72	7.19	14.99	12.77	7.59	9.64
A	1	20.06*	31.85*	44.94*	55.46*	30.23*	22.84*
B	1	0.00310	2.41	1.52	0.57	0.88	0.061
AB	1	0.00127	1.36	2.76	1.71	4.93	10.33*
A^2	1	16.52*	0.19	23.65*	5.69*	1.89	14.80*
B^2	1	4.41	0.19	0.63	0.89	0.042	0.0198
Lack of fit		3.48^{ns}	2.83^{ns}	0.12^{ns}	1.72^{ns}	0.35^{ns}	0.75^{ns}

TPC=Total phenolic content; TFC= Total flavonoid content; O. A.=Overall acceptability; *=Significant at P<0.05; ^{ns}= not significant; Df=Degree of freedom; F= ratio of variance estimates.

Effect on iron content

Figure 5 shows the response surface plot at different replacement level of wheatgrass powder and baking time on iron content. Table 3 indicated that iron content was affected by wheatgrass powder, with positive linear ($p<0.05$) and negative quadratic effects at $p<0.05$. However, the same pattern also can be observed on the positive linear and negative quadratic effects of baking time on wheatgrass cupcake (Table 3). As the wheatgrass powder replacement level and baking time increased the iron content of wheatgrass cupcake also was increased.

Effect on overall acceptability

For the evaluation of sensory attribute of formulated product, overall acceptability was considered as response variable. In this study the hedonic ratings of sensory attribute i.e. overall acceptability was observed 6.86 (like moderately) by the panelists (Table 2). Overall acceptability of the optimized product was found increase with increase in the amount of wheatgrass powder, and baking time. Figure 1 shows the response surface for the effect of independent variables on the overall acceptability of wheatgrass cupcake. As shown in Table 3, overall acceptability was negatively related to the linear and quadratic effects of wheatgrass powder ($p<0.05$) and baking time ($p<0.05$). The overall acceptability was significantly decreased with the increase level of wheatgrass powder and baking time (Figure 6). However he interaction effects of wheatgrass powder and baking time were positive at $p<0.05$ respectively shows that the moderate amount of wheatgrass powder and optimum time period seemed to be more acceptable by the panelists, and could increased the overall acceptability of wheatgrass cupcake.

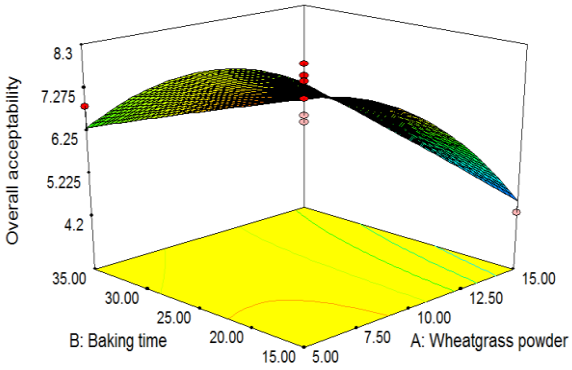

Figure 6. Response surface plot of the effects of wheatgrass powder (g) and baking time (min.) on overall acceptability of wheatgrass cupcake.

The representative antioxidant materials in wheatgrass are phenolic compounds, including flavonoids and ascorbic acid content. Significantly, these compounds have been reported to have antioxidant activity and are thought to be responsible for the antioxidant activity of backed wheatgrass cupcake samples by inhibiting free radicals (Borek 2001). However, the increased amount of wheatgrass powder and the baking time resulting in the highest antioxidant activity, including total phenolic content, total flavonoid content and DPPH radical scavenging activity and highest ion content was found when increased amount of wheatgrass powder and moderate period of baking time; however, ascorbic acid content largely increased in baked wheatgrass cupcake backed for less time period.

Other active constituents may contribute to the antioxidant activity of the backed wheatgrass cupcake. Many studies have indicated that the presence of browning products is related to increases in antioxidant activity, and browning products have been shown to exert antioxidant action by breaking the free radical chain through the donation of hydrogen atoms (Eichner. 1981;Manzocco et al.,2000). A positive and highly significant relationship between total phenolics and antioxidant activity in plant products has also been previously demonstrated (Stratil, Klejdus, and Kubán. 2006). In this study, the browning intensity gradually increased during the wheatgrass cupcake manufacturing process, and it exhibited a trend

similar to that of total phenolic content, total flavonoid content and DPPH radical scavenging activity of wheatgrass cupcake backed at various time periods temperatures. Moreover, all these properties were enhanced in backed wheatgrass cupcake backed for long period of time and at high temperatures (Nencini et al., 2011).

Optimization and characterization

In this study, Response surface methodology was used for the optimization of independent variables i.e. amount of wheatgrass powder, and baking time and their effect on responses i.e. (TPC, TFC, DPPH, ascorbic acid and Ion content) and the sensory attributes (overall acceptability). It reveals that the terms in each model had a significant effect on the responses- TPC, TFC, DPPH, ascorbic acid, Ion content and overall acceptability, suggesting a good fit of each model. The response optimization was achieved as per the desired criteria based on the acceptance of the product. The solutions could be achieved from the software with the maximum desirability as well as the acceptance and the optimum variable levels by being at random starting points and proceeding on the path of the steepest slope to a maximum. The best among them was taken as the optimum. Wheatgrass powder 5.00 g, with 35:00 minutes baking time achieving the desirability of 1 and OAA of 6.86 on nine point hedonic scale was the optimized ingredient composition with the best fit. The predicted response value of acceptability, TPC, TFC, DPPH, ascorbic acid and Ion content scores were 7.18, 14.25, 0.62, 78.30, 11.43, 11.98 as against actual values 6.86, 11.56, 0.59, 71.86, 8.56, 6.26 respectively, which were in concurrence with each other.

Proximate Composition

Nutritional composition of wheatgrass cupcake is presented in Table 5. Wheatgrass cupcake possessed good quantities of protein 12.65%, fiber 8.8%, along with minerals such as calcium 160mg/100 g, iron 12.46mg/100 g and phosphorous 86.45 mg/100 g, ascorbic acid 8.46 as compared to control. Increasing addition of wheatgrass powder (5–15%) has shown good enhancement in protein, minerals and fiber in cupcake when compared to control. Rahman and Hiregoudaret (2014) produced muffins using 2.5-7.5% of dry wheatgrass powder, and that muffin formulated with replacement of wheat flour with up to 5.0 per cent wheatgrass had higher protein and fiber content as compared to muffin prepared with 100 per cent wheat flour. This study demonstrated that wheat grass powder offers a great potential to be used in a variety of food products to enhance their nutritional quality.

Table 5.Proximate Analysis

Parameters	Control	Optimized wheatgrass cupcake
Moisture g/100g	15 ± 0.81	13.00±0.65
Protein g/100g	8.5 ± 0.84	12.65 ± 1.23
Fat g/100g	7.46 ± 0.65	5.50±1.12
Ash g/100g	2.83 ±1.24	3.50±0.65
Fiber g/100g	1.2± 0.40	8.8 ± 0.73
Carbohydrate g/100g	48.2±2.56	49.5±1.15
Phosphorus mg/100g	56.00±1.36	78.33±2.21
Calcium mg/100g	78.00±2.14	160.34±2.45
Iron mg/100g	6.70±0.20	12.46±1.36

The nutritive value of wheatgrass powder supplemented formulation was found higher than that of control product. It is clear that supplementation of the basic formula with the wheatgrass powder resulted in higher dietary fiber, and mineral matter content. This fulfills approximately one third nutritional requirement of school going children (Table 5). The fiber and minerals content was relatively high in this product, which indicates that incorporation of natural plant fibers, and their minerals in food products thus increasing the mineral and fiber consumption in daily diet.

Antioxidant analysis

Antioxidant potential of optimized formulation wheatgrass cupcake was shown in (Table 6). Wheatgrass powder supplemented optimized formulation contained higher antioxidant potential including 0.71 mmolFe(II)Eq/g FRAP value, 0.68 % Reducing capacity, and 65.65umolAAE/g Metal chelating activity than control 0.25 mmolFe(II)Eq/g FRAP value, 0.32 % Reducing capacity, 38 µmolAAE/g Metal chelating activity respectively. Incorporation of wheatgrass powder, gave an excellent antioxidant effect on the wheatgrass cupcake as compared with control. Addition of wheat grass enhanced the antioxidant effect of the optimized formulated product. The higher efficiency of the wheatgrass powder could be due to the persistence of this natural antioxidant during processing. In addition, natural antioxidants are safe and impart health benefits to the consumer.

Table 6. Antioxidant analysis

Treatments	FRAP (mmolFe(II)Eq/g)	Metal chelating (µmol/AAE/g)	Reducing powder (%)
Control	0.25±0.23	38.12±0.86	0.32±0.23
Optimised wheatgrass cupcake	0.71±0.15	65.67±0.75	0.68±0.06

Anti-nutritional analysis

The anti-nutritional factors of optimized product are summarized in (Table 7). Highest tannin, trypsin inhibitor and phytate content was found in optimized wheatgrass cupcake (0.56%, 20%, 38.67%) respectively, and lowest was in case of control (0.43%, 18%, 34.33%) respectively.

It must be noted that anti-nutritional factors (tannin, trypsin inhibitor and phytate content) of wheatgrass cupcake was found higher than control product. Studies suggest that anti-nutritional factors can be reduced by various food processing techniques.

Table 7. Anti- nutritional analysis

Treatments	**Tannin (mg/100g)**	**Phytate (%)**	**Trypsin inhibitor (%)**
Control	0.43±0.11	34.33±1.52	18.00±1.25
Optimized wheatgrass cupcake	0.56±0.21	38.67±1.52	20.00±1.41

4. Conclusions

The wheatgrass cupcake formulation can serve as a good source of dietary fiber, minerals and is a novel approach for increasing the mineral and fiber consumption in daily diet. Wheatgrass can be considered as a good source of natural antioxidants and has the potential to enhance the health benefits to the consumer.

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EFFECTS OF THYMOL SUPPLEMENTATION AGAINST TYPE 2 DIABETES IN STREPTOZOTOCIN-INDUCED RAT MODEL

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Abstract

The present investigation was aimed to study the effect of thymol, an active compound of *Nigella sativa*, on streptozotocin (STZ) induced diabetic rats. Thirty male Wistar rats were randomly divided into five groups (i) Normal control, (ii) Diabetic control, (iii) Diabetic treated with insulin (iv) Diabetic treated with thymol and (v) Normal rats treated with thymol. The rats were injected with STZ at a dose of 55 mg/kg body weight intraperitoneally to induce diabetes. Thymol was given orally at a dose of 40mg/kg body weight for 28 days. At the end of the study, the rats were sacrificed and blood samples were drawn by cardiac puncture to determine the levels of Blood glucose, Plasma Alkaline phosphatase (ALP), Total Cholesterol, Triglycerides, Low-density lipoprotein (LDL), High-density lipoprotein (HDL), Urea, Creatinine, Serum glutamic-oxaloacetic transaminase (SGOT), Serum glutamate pyruvate transaminase (SGPT), Plasma malondialdehyde (MDA) content, Advanced oxidation protein products (AOPP), Sialic acid (SA). Markers of antioxidants as Ferric reducing antioxidant power (FRAP) and erythrocyte Reduced Glutathione (GSH) were also measured. Radical scavenging activity in terms of percent 2, 2-diphenyl -1- picrylhydrazyl (DPPH). Blood glucose level, Total Cholesterol, Triglycerides, LDL, Urea, Creatinine, SGOT, SGPT, ALP, MDA, AOPP, SA levels which were found elevated due to diabetes reduced significantly to near normal. However, HDL level, % DPPH, FRAP content and erythrocyte GSH levels elevated after supplementation. We observe that administration of thymol afforded remarkable protection against diabetes and its related complications.

Key words: Thymol, *Nigella sativa,* Streptozotocin, Cholesterol.

Introduction

Diabetes mellitus is one of the most common disorders affecting almost 8.8% of the world's population. According to International Diabetes Federation (IDF) estimates there are 424.9 million adults (aged 20-79) worldwide with diabetes mellitus (DM), a number that is expected to reach to 628.6 million by 2045 (IDF, 2017).

Globally, diabetes is one of the six major causes of death (Petchi *et al.,* 2014). Medical interventions to manage diabetes include glucose-lowering agents such as alpha-glucosidase inhibitors, biguanides, sulfonylureas and thiazolidinediones and sometimes insulin. All these interventions are associated with severe to mild side effects after a given amount of time. These compulsions provide a perfect setting for use of alternative systems of medicine based on traditional knowledge (Parasuraman *et al.,* 2010).

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Plants and their active compounds have frequently been used for medicinal purposes. According to the World Health Organization (WHO), "a medicinal plant is a plant which, in one or more of its organs, contains substances that can be used for therapeutic purposes, or which are precursors for chemo-pharmaceutical semi-synthesis." Such plants are in great demand by pharmaceutical companies for their active ingredients (Huai *et al.,* 2010, Husain *et al.,* 2008).

In the Indian traditional system of medicine, *Nigella sativa*, an annual herb belonging to Ranunculaceae family have been used for thousands of years as a spice and food preservative to a variety of food products as bread, yogurt, pickles, sauces, salads etc. (Hajhashemi *et al.,* 2004). *Nigella sativa* is traditionally used for its galactagogue, appetizer, thermogenic and diuretic effects (Hosseinzadeh *et al.,* 2013). Furthermore, *Nigella sativa* possesses anti-microbial, anti-fungal, anti-oxidative and

anti-cancerous properties (Salem and Hossain, 2000, Salomi *et al.,* 1992). *Nigella sativa* seeds contain several bioactive compounds, one of them is thymol (THY).

Thymol (2-isopropyl-5-methylphenol) is a dietary monoterpene phenol exhibiting multiple biological activities including antioxidant (Aeschbach *et al.,* 1994) and free radical scavenging properties (Fujisawa *et al.,* 1992). The present study is undertaken to investigate the anti-diabetic potential of thymol on STZ-induced diabetes in rats.

Materials and Methods

Reagents and chemicals

Thymol was procured from Sigma Aldrich, India. All other chemicals were of analytical grade and made available from Merck, India and Himedia Labs, India.

Experimental animals and STZ induction

The experiment was carried out with 30 male Wistar rats (5 ± 0.5 months and body weight 150 ± 20 g). They were housed in a temperature controlled room (25 ± 5°C) with12-h. light-dark cycles for at least 1 week. All rats were fed with a normal laboratory diet of nutrient rich pellets containing total energy as fat, protein and carbohydrates and had free access to drinking water throughout the period of the experiment.

Experimental design

Rats were divided into five groups of six rats each and were given the following treatments:

Group I: Normal Control (NC) receiving no treatment.

Group II: Diabetic Control (DC), rats were injected single dose of STZ (55 mg/kg body weight) intra-peritoneally.

Group III: STZ induced diabetic rats were supplemented with insulin (D+INS).

Group IV: STZ induced diabetic rats were supplemented with thymol via gavage technique (oral route) at 40mg/kg body weight/day for 28 days (D+THY).

Group V: Normal rats were given only thymol (40 mg/kg body weight/day for 28 days) (N+THY).

Diabetes was induced by intra-peritoneal injection of STZ at a dose of 55 mg/kg body weight. After 96 hours diabetes was confirmed by the determination of fasting blood glucose level with the help of a glucometer, rats with blood glucose (>250 mg/dl) were considered diabetic and included in the study. All treatment was carried out up to 28 days. The animals of the first group were simultaneously administered water until 28 days.

At the end of the experimental period, the rats were fasted for overnight (12 hours) and sacrificed under light anaesthesia. Blood samples were collected by cardiac puncture into 10 unit/ml heparin rinsed anticoagulant syringes. Plasma was obtained from blood sample after centrifugation (1500×g for 10 min) and stored at 4°C for analysis. After the removal of plasma (immediately frozen at -80°C until use for biochemical assays), buffy coat and the upper 15% of packed red blood cells (PRBCs), the RBCs were washed twice with cold phosphate buffered saline (PBS) (0.9% NaCl and 10 mmol/l Na_2HPO_4, pH 7.4) and then used for experiment. All protocols for experiments were approved by the Animal Care and Ethics Committee of University of Allahabad.

Biochemical assays

Blood glucose values were determined using an Accu-Check Active Glucometer (Roche Diagnostics, Mannheim, Germany). Lipid profile (Total Cholesterol, HDL, LDL), SGOT, SGPT, urea, creatinine and alkaline phosphatase were measured using reagent kits from Erba Diagnostics, Mannheim, Germany.

Measurement of total antioxidant activity by FRAP

The total antioxidant potential of the plasma samples was determined using a modification of the ferric reducing ability of plasma (FRAP) assay of Benzie and Strain (1996). FRAP reagent was prepared from 300 µmol/l acetate buffer, pH 3.6, 20 µmol/l ferric chloride and 10 mmol/l TPTZ made up in 40 µmol/l hydrochloric acid. All three solutions were mixed together in the ratio 10:1:1 (v:v:v) respectively, 3 ml of FRAP reagent was mixed with 100 µl of plasma and the contents were mixed thoroughly. The absorbance was read at 593 nm at 30 s intervals for 4 min. Aqueous solution of known Fe(II) concentration in the range of 100-1000 µmol/l was used for calibration. Regression equation of the FRAP values (µmol Fe(II)/l) of the plasma was used for calculation.

Determination of DPPH radical scavenging activity in plasma

This assay was performed according to the method proposed by Szabo *et al.,* (2007). 100 µl of plasma was added to 10 mmol phosphate buffer (1.9 ml) and 0.1 mmol 2, 2-diphenyl-1-picrylhydrazyl (DPPH) in methanol (2.0 ml) with a control having 2 ml of 10 mmol phosphate buffer with the same amount of DPPH solution. It was kept for incubation for 30 min at 21°C and centrifuged for 5 min at 1000×g. Absorbance was measured at 517 nm with methanol as a blank. Values were compared for control (A_0) and plasma (A) and percent radical scavenging activity was calculated by using 100 $(A_0 - A)/A_0$.

Determination of erythrocyte reduced glutathione (GSH)

Erythrocyte GSH was measured following the method of Beutler (1984). The method is based on the ability of the SH group to reduce DTNB and form a yellow coloured anionic product whose optical density is

measured at 412 nm. Concentration of GSH is expressed in mg/ml packed RBCs.

Determination of plasma advanced oxidation protein products (AOPPs)

Determination of AOPPs in plasma was based on spectrophotometric detection according to Witko-Sarsat *et al.,* (1996). Briefly, 200 ml of plasma (diluted 1:5 with phosphate buffer saline (PBS) as test), 200 ml of chloramine-T solution (0-100) µmol/l for calibration and 200 ml of PBS as blank were applied. 10 µl of 1.16 kol potassium iodide and 20 µl of acetic acid were added and absorbance at 340 nm was measured immediately. Concentration of AOPPs was expressed as µmol/l of chloramine-T equivalents.

Determination of plasma Sialic acid (SA) level

Sialic acid was determined by the method of Spyridaki *et al.,* (1996). Briefly, 0.5 ml of plasma was treated with 0.10 ml of 0.04 mol periodic acid. It was mixed thoroughly and allowed to stand in ice bath for 30 min. Thereafter 1.25 ml of resorcinol working solution (5 ml of 6.0% resorcinol solution, 0.125 ml of 0.1 mol copper sulphate solution and 19.875 ml of distilled water, brought to a final volume of 50 ml with 10 mol HCl) was added, mixed and heated at 98°C for 5 min. Thereafter cooled in an ice bath for approximately 2 min. Lastly 3.25 ml of n-butanol was added and mixed well. Placed in a water bath at 37°C for 3 min for stabilizing the colour. Their absorbance was measured at 625nm against a reagent blank. Plasma Sialic acid is measured as µmol.

Determination of lipid peroxidation (malondialdehyde (MDA) level) in plasma and erythrocytes

Plasma or erythrocyte MDA was measured according to the method of Esterbauer and Cheeseman (1990) with slight modification. Packed RBC (0.2 ml) was suspended in 3 ml PBS containing 0.5 mmol/l glucose, pH 7.4. The lysate (0.2 ml) was added to 1 ml of 10% trichloroacetic acid (TCA) and 2 ml of 0.67% thiobarbituric acid (TBA) boiled for 20 min at 90–100°C, cooled and then the mixture was centrifuged at 1000×g for 5 min and the absorbance of supernatant was read at 532 nm. The concentration of MDA in erythrocytes was calculated using extinction coefficient ($\varepsilon = 31,500$) and is expressed as nmol/ml of packed RBC.

Statistical analysis

Statistical analysis was performed using one-way ANOVA followed by Tukey's Multiple Comparison Test with Graph Pad PRISM Software, San Diego, CA, USA, version 5.01 for Windows. All values are expressed as the mean ± SD. All the values with $P<0.05$ were considered as statistically significant.

Results and Discussion

Diabetes mellitus is a pathologic condition, resulting in severe metabolic imbalance and non-physiologic changes in many tissues more particularly in pancreas, where oxidative stress plays an important role in the etiology (Baynes and Thorpe, 1996). Diabetics and experimental animal models exhibit high oxidative stress due to persistent and chronic hyperglycemia which promotes free radical generation (Baynes and Thorpe, 1996). In diabetes mellitus, alterations in the endogenous free radical scavenging defence mechanisms may lead to ineffective scavenging of reactive oxygen species, resulting in oxidative damage and tissue injury. Ihara *et al.,* (1999) examined oxidative stress markers in experimental diabetic rats and found increased reactive oxygen species (ROS) in pancreatic islets. Free radicals may play an important role in the development of late complications of diabetes mellitus (Mohamed, 1999).

In this study, the anti-hyperglycemic effect of the thymol supplementation was investigated using STZ induced diabetic rats. After 28 days of oral administration of thymol, significant reduction in blood glucose levels was observed in the STZ-induced diabetic rats. The blood glucose was significantly elevated ($P<0.01$) in diabetic rats as compared to normal control rats (fig. 1). In diabetic rats, oral administration of thymol (40 mg/kg body weight) lowered the blood glucose significantly ($P<0.01$). Our findings agree with the findings of Saravanan and Pari (2015), who observed that thymol treated group showed significantly decreased blood glucose levels (Fig. 1).

The lipid profiles and hepatic parameters of control and treated rats were evaluated (Table 1). Table 1, shows an increase in levels of Total cholesterol (56%), Triglyceride (52%) and LDL-Cholesterol (199%) as well as a decrease in HDL-Cholesterol (31%) of diabetic rats as compared to the control group. In addition, data also showed a significant ($p<0.05$) increase in the activities of SGOT (61%), SGPT (240%) and ALP (116%) in diabetic rats as compared to the control rats, suggesting hepatocellular damage as a result of STZ toxicity. These observed increased levels in diabetic rats may be explained due to oxidative damage caused by the diabetogenic agent STZ. It was also suggested that hepatic damage may cause this abnormal rise in lipid profile parameters and liver enzymes levels (Asante *et al.,* 2016). Interestingly, the thymol administration showed an important protective action in diabetic rats by reducing the hepatic toxicity. Indeed, thymol treatment significantly reduced the SGOT ($p<0.05$), SGPT ($p<0.01$) and ALP ($p<0.001$) enzyme activities and the obtained values were nearly similar to those of the control rats. Furthermore, as shown in table

Table 1: Effect of Thymol supplementation on biochemical profile of STZ-induced diabetic rats.

S. No.	Parameters	Normal Control	Diabetic Control	Diabetes + Insulin	Diabetes + Thymol	Normal + Thymol
1.	Total Cholesterol (mg/dl)	93±7	145 ± 16***[a]	119± 8***[b]	120 ± 8***[b]	90 ± 5
2.	HDL-C (mg/dl)	48 ± 5	33 ± 7**[a]	35± 8[b]	37 ± 8[b]	44 ± 4
3.	LDL-C (mg/dl)	40.36 ± 8	120.53±9***[a]	87.55± 9***[b]	80.55 ± 9***[b]	33.50 ± 6
4.	Triglyceride (mg/dl)	89 ± 7	135 ± 15***[a]	107± 9***[b]	107 ± 9***[b]	85 ± 11
5.	SGOT (U/L)	105 ± 9	169 ± 5***[a]	137± 12*[b]	132 ± 6*[b]	111 ± 7
6.	SGPT (U/L)	5.59 ± 1.49	19.03±.07***[a]	11.47±2.2***[b]	13.47 ± 2.21**[b]	4.47 ± 0.92
7.	Alkaline Phosphatase (U/L)	252.56± 9.12	545 ± 37.27***[a]	349± 30***[b]	392 ± 30.21***[b]	261 ± 24.59
8.	Urea (mg/dl)	39.90 ± 4.38	61.24 ± 7.34***[a]	52± 5.89[b]	50 ± 5.89*[b]	43 ± 4.03
9.	Creatinine (mg/dl)	0.65 ± 0.08	1.34 ± 0.218***[a]	0.82± 0.09***[b]	0.67 ± 0.09***[b]	0.59 ± 0.03

Values represent mean ±SD for 6 rats. *p < 0.05, **p < 0.01, ***p < 0.001.
[a]Diabetic group as compared to control group, [b]Experimental group as compared to Diabetic control.

1, the plasma lipid profile progressed to the normal values of control rats which are in agreement with reported findings (Asante *et al.,* 2016). It is also important to note that thymol effect on the hepatic dysfunction parameters and lipid profile was similar to that obtained by standard insulin (Table 1).

Table 1, presents the kidney toxicity indices of control and treated rats. The obtained results showed that hyperglycemia increased the urea and creatinine levels by 53 and 106%, respectively in diabetic rats as compared to control group. Thymol administration to diabetic rats improved the indices related to kidney dysfunction induced by diabetes. In fact, thymol administration to diabetic rats significantly decreased the urea ($p<0.05$) and creatinine ($p<0.001$) levels by 10% and 38%, respectively, which is similar to other studies (Jdir *et al.,* 2017). As compared to normal individuals, high urea and creatinine levels represent important kidney dysfunction markers (Liu *et al.,* 2006). Thus, it can be concluded that diabetic rats suffer from renal disorders due to the protein glycation that lead to the muscle loss as well as to the increase of purine release, which is the main uric acid source (Jagdale *et al.,* 2016). The alleviation of kidney dysfunction parameters could be explained by the attenuation of oxidative stress situation *via* glucose level regulation.

Oxidative stress is an imbalance between reactive oxygen species (ROS) production and the antioxidant defence systems (Favier, 1997). The measure of plasma anti-oxidant capacity revealed a good association of oxidative stress in several pathologies and particularly, in the diabetes mellitus (Bonnefont-Rousselot *et al.,* 2000). To combat ROS, the organism has enzymatic defence mechanisms as superoxide dismutase, glutathione peroxidase or catalase and non-enzymatic types as glutathione and α-tocopherol. The ROS scavenging capacity through the antioxidant systems becomes insufficient in diabetes and a constant oxidative stress develops (Ohkawa *et al.,* 1979). Due to the large number of antioxidants present in plasma, several methods have been developed: ferric reducing antioxidant power (FRAP), DPPH radical scavenging activity and Reduced glutathione (GSH) level (Janaszewska and Bartosz, 2002, Huang *et al.,* 2005).

In our study, plasma FRAP value significantly depleted ($p<0.001$) in STZ-induced diabetic control rats as compared to normal control rats. After thymol supplementation, FRAP value significantly ($p<0.001$) improved in STZ-induced diabetic rats as compared to diabetic control rats (Fig. 2). These findings are in concordance with the study of Sasvari and Nyakas (2003). Similar results were found with percent radical scavenging activity (Fig. 3) and reduced glutathione level (Fig. 4) in our study as well as studies of Meziti *et al.,* (2012).

Reduced radical scavenging activity of plasma causes oxidative modulation of proteins which may be one of the reasons of altered physiological processes in type 2 diabetic patients (Pandey *et al.,* 2010). Therefore, the measurement of the protein oxidation is a clinically important factor for the prediction of the diabetes or degree of oxidative stress in diabetes and stress-related diseases. Advanced protein oxidation products (AOPP) are defined as dityrosine containing crosslinked protein products due to action of chloraminated oxidants, mainly hypochlorous acid and chloramines, produced by myeloperoxidase in activated neutrophils. It is considered a reliable marker for estimating the degree of protein oxidative modification (Witko-Sarsat *et al.,* 1996). Oxidation of proteins can lead to a whole variety of amino acid modifications, it may be selective and specific. Accumulation of protein products is associated with a number of diseases, including coronary artery diseases (Kaneda *et al.,* 2002), diabetes (Martin-Gallan *et al.,* 2003), preterm neonates (Buonocore *et al.,* 2002) and dentritic cell stimulation (Witko-Sarsat *et al.,* 1998). In our study, a significant ($P<0.001$) elevation in AOPP level

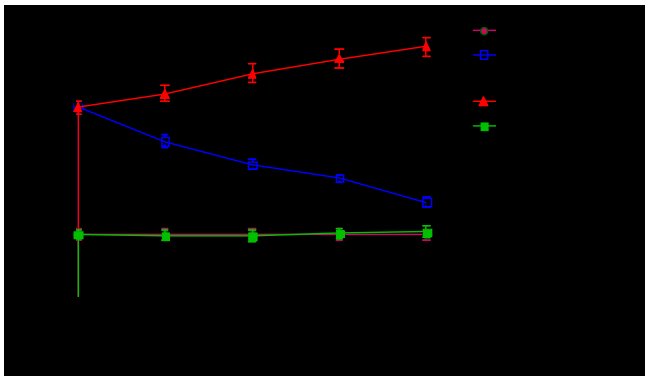

Fig. 1: Effect of Thymol Supplementation on Blood Glucose Level of STZ- induced Diabetic Rats.

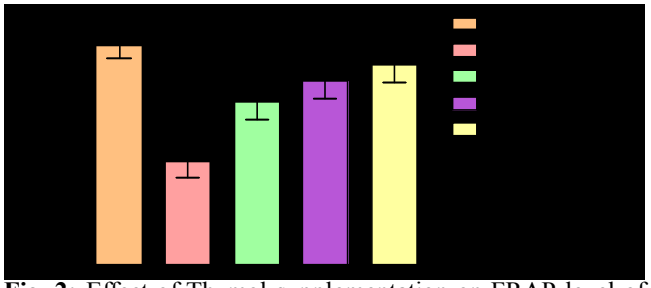

Fig. 2: Effect of Thymol supplementation on FRAP level of STZ-induced diabetic rats. FRAP value is expressed in µmolFe(II)/l plasma. Values represent mean ±SD. ***($p < 0.001$) as compared to Diabetic control.

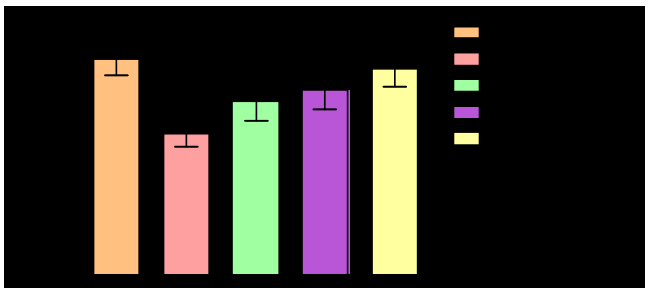

Fig. 3: Effect of Thymol supplementation on DPPH % inhibition of STZ-induced diabetic rats. Values represent mean ±SD. ***($p < 0.001$) as compared to normal control. **($p < 0.01$) as compared to diabetic control. *($p < 0.05$) as compared to diabetic control.

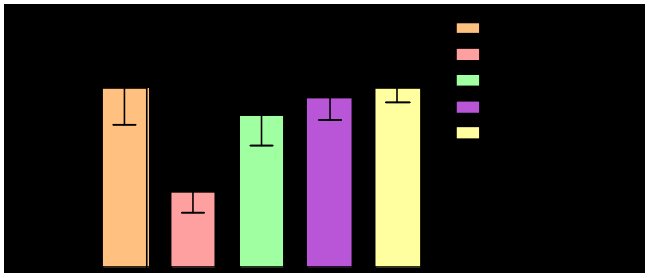

Fig. 4: Effect of Thymol supplementation on Reduced glutathione (GSH) level of STZ-induced diabetic rats. GSH value is expressed in mg/ml of packed erythrocytes. Values represent mean ±SD. ***($p < 0.001$) as compared to diabetic control.

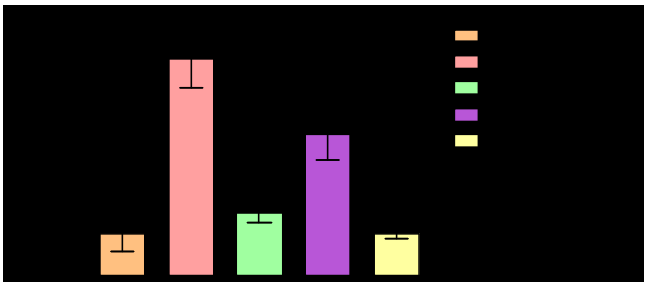

Fig. 5: Effect of Thymol supplementation on AOPP level of STZ-induced diabetic rats. AOPP value is expressed in µmol/l of plasma. Values represent mean ±SD. ***($p < 0.001$) as compared to diabetic control.

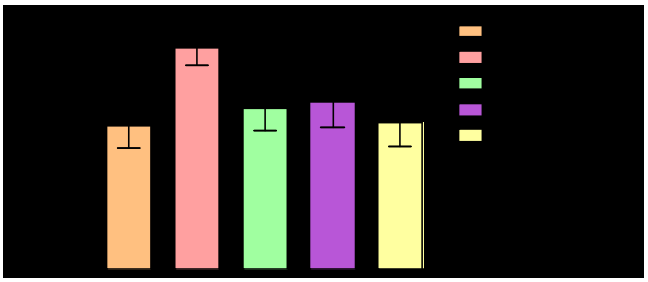

Fig. 6: Effect of Thymol supplementation on Sialic acid content of STZ-induced diabetic rats. Sialic acid value is expressed in µmol of plasma. Values represent mean ±SD. ***($p < 0.001$) as compared to diabetic control. **($p < 0.01$) as compared to diabetic control.

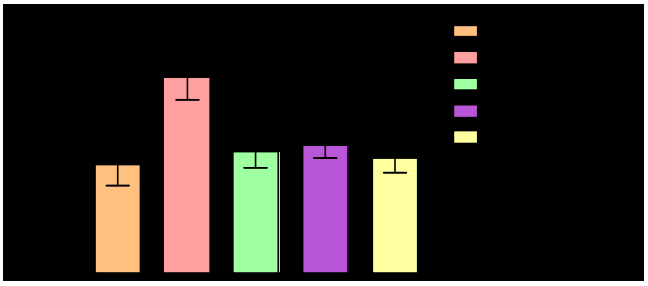

Fig. 7: Effect of Thymol supplementation on plasma malondialdehyde (MDA) content of STZ-induced diabetic rats. Concentration of MDA is expressed as nmol/ml of plasma. Values represent mean ±SD. ***($p < 0.001$) as compared to diabetic control.

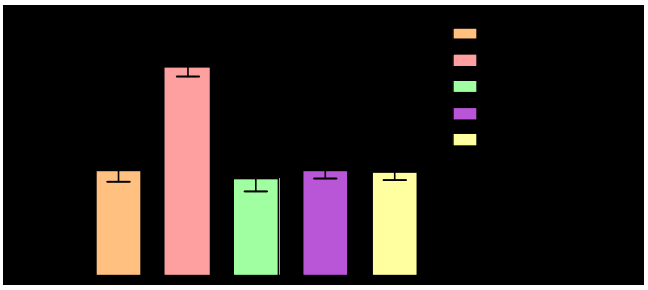

Fig. 8: Effect of Thymol supplementation on erythrocyte malondialdehyde (MDA) content of STZ-induced diabetic rats. Concentration of MDA is expressed as nmol/ml of packed erythrocytes. Values represent mean ± SD. ***($p < 0.001$) as compared to diabetic control.

was observed in diabetic group as compared to control, which significantly (P<0.01) decreased after the administration of thymol (Fig. 5). This study showed that thymol is as effective as insulin in decreasing the levels of AOPP value and blood glucose level in STZ induced diabetic rats.

Sialic Acid (SA) is an acetylated derivative of neuraminic acid and is an essential component of glycoproteins and glycolipids. Vascular endothelium carries a high concentration of Sialic acid where it governs permeability. It is necessary for the cell-surface residency of platelet and promotes endothelial barrier integrity (Cioffi *et al.,* 2012). It also acts as a co-factor of many cell receptors and is positively associated with most of the serum acute phase reactants. In diabetic state, extensive micro vascular damage sheds Sialic acid into circulation (Prakash and Sudha, 2013). Several studies have highlighted that Sialic acid metabolism is drastically altered in diabetic condition. Such an elevation of Sialic acid level in the plasma leads to complications like retinopathy, nephropathy and neuropathy. A study of Prajna *et al.,* (2013) states that increased SA is a potential risk factor for development of nephropathy in diabetic patients. Similarly raised levels of serum Sialic acid are implicated in cardiovascular diseases. In our study, a significant elevation (P<0.001) in plasma Sialic acid was observed in diabetic control rat. Oral administration of thymol to STZ induced diabetic rat restored the levels of Sialic acid in plasma to near normal (Fig. 6).

Lipid peroxidation (LPO) is a well-established mechanism of cellular injury and is used as an indicator of oxidative stress in cells and tissues. Measurement of malondialdehyde has been used as an indicator of lipid peroxidation. LPO is the process of oxidative degradation of polyunsaturated fatty acids and its occurrence in biological membranes causes impaired membrane function, structural integrity, decrease in membrane fluidity and inactivation of several membrane bound enzymes (Pandey and Rizvi, 2010). Under oxidative stress, the erythrocyte membrane is prone to lipid peroxidation that involves cleavage of polyunsaturated fatty acids at their double bonds, leading to the formation of MDA, an increased MDA content is an important indicator of lipid peroxidation. Increased level of MDA in erythrocyte has been reported in many disease conditions which are accompanied with oxidative stress (Bhatia *et al.,* 2003). In this study, plasma and Erythrocytes MDA content was significantly (p<0.001) increased in the STZ induced diabetic rats as compared to normal control rats and in contrast, MDA level was significantly decreased (p<0.001) in diabetic rats when supplemented with thymol (Fig. 7 and 8). This toxicity may be due to the alterations in membrane integrity *via* the formation of reactive oxygen species by successive hydroperoxide formation and β cleavage of polyunsaturated fatty acids *in vivo* or due to perturbation of antioxidant defence mechanisms.

Conclusion

Results obtained in the present study suggest that thymol, an active constituent of *Nigella sativa* has an overall protective effect against STZ induced diabetes mellitus in a rat model. Thymol produces significant anti-hyperglycaemic effect in diabetic rats. We suggest that these results provide strong evidence in support of the pharmacological use of thymol as an anti-diabetic complement in cases of type 2 diabetes.

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The Antioxidant Efficacy of Wheatgrass (*Triticum aestivum*) on Mercuric Chloride ($HgCl_2$) -Induced Oxidative Stress in Rat Model

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Abstract

Mercury is a harmful toxic pollutant, which has hepato-nephrotoxic, hematotoxic, genotoxic and neurotoxic effects. The aim of the study was to evaluate the protective efficacy of wheatgrass on mercuric chloride ($HgCl_2$) induced oxidative stress and associated complications in rat model. Albino rats were divided into four groups (three rats per group). Group I normal control group. Group II oxidative stressed group received mercuric chloride (0.5 mg/kg/day). Group III only received wheatgrass extract (100 mg/kg/day), whereas Group IV received wheatgrass (100 mg/kg/day) after one hour, followed by mercuric chloride (0.5 mg/kg/day) for 30 days. The results of the study showed that wheatgrass supplementation significantly decreased the $HgCl_2$ induced elevated oxidative stress parameters Plasma Malondialdehyde (MDA) content, Plasma membrane redox system (PMRS), Advanced oxidation protein products (AOPP), simultaneously elevated lipid profile (Total Cholesterol, Triglycerides, Low-density lipoprotein (LDL), liver enzymes as, Plasma Alkaline phosphatase (ALP), Aspartate aminotransferase (AST), and Alanine aminotransferase (ALT), Serum Urea, and Creatinine levels in rats. In addition, wheatgrass treatment improved the antioxidant status in terms of intracellular Reduced Glutathione (GSH), Ferric reducing antioxidant power (FRAP) and 2, 2- diphenyl -1- picrylhydrazyl (DPPH). Therefore it can be concluded that wheatgrass has great potential to diminish the stress-mediated complications and improve the antioxidant status.

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Introduction

Exposure of mercury pulled attention of whole world due to its harmful effects. It is a heavy metal and harmful toxic pollutant which has multiple adverse effects. It is commonly used in battery monometer, dental amalgamation, electrical switches, as a catalyst in chemical manufacturing, wood preservative, photography industry, limited use in gold mining and in leather industry although. Furthermore it is heavily used in many industries household product, pharma industry and agriculture industry,[1] which leads to high dispersion in environment. Bringing in notice over there fatal effect on human health and the environment.[2-6] Its effects are nephrotoxic,[7, 8] hematotoxic,[6] hepatotoxic,[8, 9] genotoxick,[10] neurotoxic[11] and reduced reproductive strength.[12, 13]

Mercury affects antioxidant mechanisms and increase in oxidative stress through the alteration in various mechanism, is possibly resulted in the reduction in Adenosine triphosphate (ATP) content, decrease of antioxidant enzyme activity, depletion of cellular cysteine thiols, mercury has ability to react with Glutathione (GSH, thiol antioxidant)[14] and creates a complex formulation which reduced the antioxidant GSH level in body and results to oxidative stress.[15, 16] Oxidative stress leads to impaired transport mechanism, lipid peroxidation, denaturation of protein and DNA.[17-20]

Various previous studies reported that plant and plant based products are good source of natural antioxidant that can be used as herbal natural remedy for protection from oxidative stress, plant based treatments has no side effects and has been used since long time.[21] Among the various medicinal plants wheatgrass is one which is good source of antioxidant and used globally in various perspectives. Wheatgrass (*Triticum aestivum*) is a young grass (seven to nine days) of wheat plant. Wheat (*Triticum*) species is an annual and biennial cereal grass of the (Poaceae) family, is the world's largest edible grain cereal grass crop which is widely cultivated almost all over the world. Almost more than twenty five cultivars of wheat are identified in world of which *Triticum aestivum* is the foremost Indian cultivar of wheatgrass.[22] Wheatgrass is a versatile medicinal plant and had been used as a natural medicinal plant from the ancient period of time. In traditional Indian ayurvedic system wheatgrass is an excellent source of vitamins (A, C, E), minerals (iron, calcium, phosphorus), proteins, enzymes, chlorophyll and other bioactive compounds (Rutin, Chlorophyllin, Apigenin, Quercetin).[23] These nutrients and bioactive compounds increased clinical utility of wheatgrass and makes it a medicinal plant for the treatment of various diseases and life threatening conditions. It has been shown various pharmacological potentials such as anti-cancer activity,[24] anti- ulcer activity,[25] anti -diabetic activity,[26] antioxidant activity,[27] anti- thalassemic activity[28] anti- arthritic activity, anti- inflammatory and anti- aging activity.[29] These pharmacological potentials were due to presence of various phenolic, flavonoid compounds, vitamins and minerals that makes wheatgrass a sturdy natural therapeutic agent for the prevention of oxidative stress and allied complications with mercuric chloride toxicity. Therefore, the purpose of the experiment was to assess the protective effect of wheatgrass on mercuric chloride induced oxidative stress in rats.[1]

Materials and Methods

Chemicals and Reagents

Mercuric chloride ($HgCl_2$, 99.5% purity) was purchased from Sigma-Aldrich Chemical Company India and other required chemicals of analytical grade were procured, Merck India and HIMEDIA Labs, India.

Cultivation of the Wheatgrass

Wheatgrass seeds (cultivar sharbati) were purchased from local market of Allahabad, India. For experimental work wheatgrass was grown at laboratory of Centre of Food Technology, University of Allahabad, U.P. India, by using try method in indoor conditions. Plastic trays were filled with soil contains three parts of soil and one part of organic fertilizer. One night soaked wheat grains were then evenly spread on the surface of the soil and further covered with a thin layer of soil. Spray some water evenly over soil and three to four hours morning sunlight was allowed daily for growth of grass. On the seventh day, grass was harvested. At this stage, wheatgrass is at its nutritional peak.[30]

Preparation of Plant Extract

The harvested wheatgrass was shorted and cleaned with water, and then dried in a cabinet tray dryer (Chemida, Mumbai, India) at 55 ± 2°C for six hours. After then dried grass was subjected to making

fine powder by using a high speed electronic mixer grinder (Sumeet Domestic Plus, M/s. Sumeet, Nashik, India), and passed through fine sieve no. 40. 10g wheatgrass powder was suspended in 100 ml of ethanol using 250 ml conical flask and kept on orbital shaker for 48 h at 37°C. After 48h, the supernatant was filtered through Whatman filter paper no.1 and lyophilized the sample for drying. The filtrate was then reduced to 1/10th of its initial volume. Obtained lyophilized dried sample was then dissolved in drinking water for dosing and stored at 4°C for further analysis.[31, 32]

Experimental Animals

The experiment was carried out with 12 male albino rats (4 to 5 months old) with body weight between 157 ± 51g (IAEC/AU/2017(1)/008). They were housed in a temperature controlled facility (25 ± 5°C) with 12 h light–dark cycle for one week, at laboratory of Department of Biochemistry, University of Allahabad, U.P. India. All rats were fed with normal laboratory diet nutrient rich pellets, and had free access to drinking water.

Animal Model and Study Protocol

After the one week of stabilization period, the rats were randomized and grouping three rats were placed in each group, and were given the following dosing:

Group I

Normal control (NC), received no treatment/ supplementation.

Group II

Oxidative stressed group (Hg), received $HgCl_2$ (0.5 mg/kg).

Group III

(Hg+WG) group received $HgCl_2$ 0.5 mg/kg body weight and wheatgrass extract 100 mg/kg body weight.

Group IV

Normal control group treated with only wheatgrass extract (N+WG) 100 mg/kg body weight.

One hour before treatment with wheatgrass, group III received $HgCl_2$. All the administrations were performed once daily through oral gavage for 30 days. Oxidative stress was induced by administration of mercuric chloride in drinking water at a dose of 0.5mg/kg body weight. The doses of $HgCl_2$ and wheatgrass were selected based on previous studies.[33, 34]

Biochemical Assays

After 24 hours of last administration. The rats were sacrificed under light anaesthesia. Blood samples were collected by cardiac puncture into 10 units / ml heparin rinsed anticoagulant syringes. Plasma was obtained from blood sample after centrifugation (1500×g for 10 min) and stored at 4°C for analysis.and then red blood cells were pelleted by centrifugation at 800 g for 10 min at 4°C. After the removal of plasma (immediately frozen at -80°C until use for biochemical assays), buffy coat, and the upper 15% of packed red blood cells (PRBCs), the RBCs were washed twice with cold phosphate buffered saline (PBS) (0.9% NaCl and 10 mmolL^{-1} Na_2HPO_4; pH 7.4) and then used for further experiment.[35]

Oxidative Stress Markers

Erythrocyte Malondialdehyde (MDA) was measured using the thiobarbituric acid (TBA) method.[36] Estimation of Advanced oxidation protein products (AOPP) levels in plasma was performed by spectrophotometric detection method.[37, 38] The activity of the erythrocyte Plasma membrane redox system (PMRS) was measured by the reduction of ferricyanide the method.[39]

Antioxidant Status

Glutathione (GSH) was estimated by 5,5'-dithiobis (2- nitrobenzoic acid) (DTNB) method it is based on the ability of the SH group to reduce DTNB and form a yellow coloured anionic product whose optical density is measured at 412 nm>[40, 41] The Ferric reducing antioxidant activity of the plasma samples was determined using Ferric reducing antioxidant power (FRAP) assay.[42, 43] Radical scavenging capacity of plasma samples were estimated by 2, 2- diphenyl -1- picrylhydrazyl (DPPH) reduction assay.[44, 45]

Lipid Profile, Liver and Kidney Functions Tests

Lipid profiles (Total cholesterol, triglyceride and LDL-Cholesterol), liver function parameters such as (Plasma Alkaline phosphatase (ALP), Aspartate aminotransferase (AST), and Alanine aminotransferase (ALT) were measured in serum

using kits from (Erba Diagnostics, Mannheim, Germany). Kidney dysfunction test (Serum Urea and Creatinine) were assayed using the kit from (Span Diagnostic Ltd.Surat, India).[46, 47]

Statistical Analysis

Statistical analyses were performed using one way ANOVA followed by Tukey's Multiple Comparison Test. P< 0.05 was considered to be statistically significant with Graph Pad Software, San Diego California USA version 5.01 for Windows. Results were expressed as the mean ±S.D.

Results and Discussion

Mercuric chloride is the one of the most harmful toxic pollutant of the world, excess accumulation of mercuric chloride in human body poses various adverse changes. It leads to formation of free radicals that cause oxidative stress and other associated complications such as hepato-nephrotoxicity,[48-50] mental retardation[51] and heart problems.[52]

In this study, protective role of wheatgrass extract was investigated in rat model against oxidative stress induced by $HgCl_2$. Wheatgrass aqueous extract act as a quencher of free radicals and neutralizing those.[14, 53] Therefore, it is concluded that the wheatgrass reduces the adverse consequences of $HgCl_2$ induced oxidative stress through countering the excessive radicals of all major types. The present study demonstrated that after 30 days of oral administration of wheatgrass extract, the antioxidant status (GSH, FRAP, DPPH) of stressed rats were significantly elevated. On the other hand reduction in lipid profiles (TC,TG,LDL), kidney function tests (urea, creatinine), liver function tests (ALP, AST, ALT) and oxidative stress markers (MDA, AOPP, PMRS) was observed in the $HgCl_2$ -induced oxidative stressed rats. The results of the study revealed that wheatgrass protects the antioxidant defense system by activating antioxidant enzymes and reduced mercury-induced oxidative stress by scavenging free radicals *in-vivo* conditions.

Evaluation of the plasma antioxidant capacity (GSH, FRAP and DPPH) is the primary step in the prophecy of oxidative stress in various pathological conditions such as heart diseases, hepato- nephrotoxicity, cancer, Alzheimer disease and hyperglycemia.[54] The result of the experiment shows that, the reduced glutathione (GSH), FRAP and DPPH levels significantly decreased in $HgCl_2$-treated rats in comparison to the normal control rats ($P < 0.001$) Figure 1,2,3. Conversely finding of the study showed that oral supplementation with wheatgrass extract significantly increased ($P < 0.001$) the antioxidant status (GSH, FRAP and DPPH) level as noticed in the Hg+WG treated groups in comparison to $HgCl_2$-treated rats and this might be as a result of the wheatgrass extract containing polyphenols, flavonoids, chlorophyll, vitamin A, vitamin C, and vitamin E, all these bioactive compounds poses good antioxidant capacity and makes wheatgrass a strong antioxidant agent.[27,55]

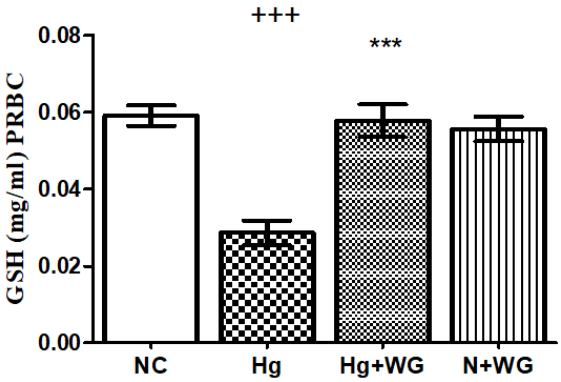

Fig.1: Effect of wheatgrass treatment on GSH level of $HgCl_2$ induced oxidative stressed rats. Concentration of GSH is expressed as mg/ml PCRB. Values are presented as means ± SD. +++P<0.001 compared with oxidative stressed group vs. control. ***P<0.001 compared with Hg+ WG vs. oxidative stressed group. (NC = Normal control, Hg = Mercuric chloride, WG = Wheatgrass)

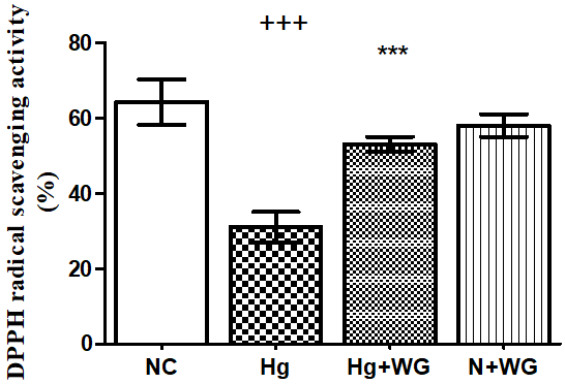

Fig. 2: Effect of wheatgrass treatment on DPPH % inhibition of $HgCl_2$ induced oxidative stressed rats. Values are presented as means ± SD. +++P<0.001 compared with oxidative stressed group vs. control. ***P<0.001 compared with Hg+ WG vs. oxidative stressed group. (NC = Normal control, Hg = Mercuric chloride, WG = Wheatgrass)

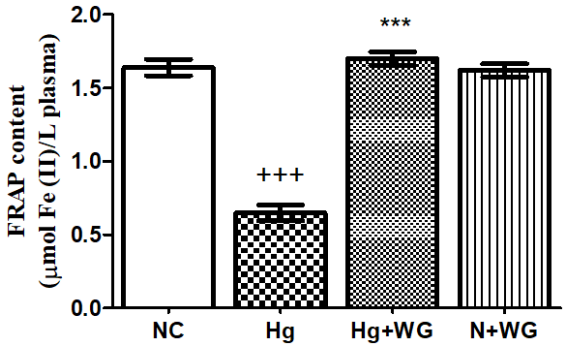

Fig. 3: Effect of wheatgrass treatment on FRAP level of $HgCl_2$ induced oxidative stressed rats. FRAP value is expressed in µmolFe(II)/l plasma. Values are presented as means ± SD. +++P<0.001 compared with oxidative stressed group vs. control. ***P<0.001 compared with Hg+ WG vs. oxidative stressed group. (NC = Normal control, Hg = Mercuric chloride, WG = Wheatgrass)

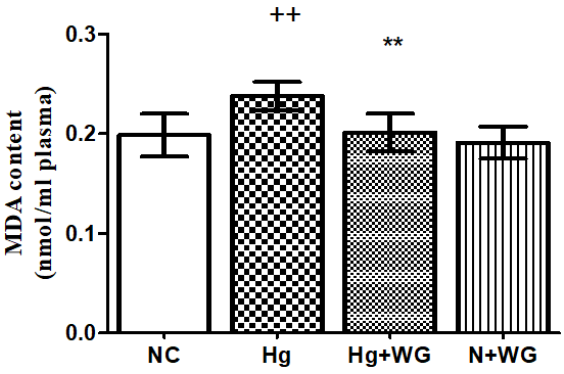

Fig. 4: Effect of wheatgrass treatment on plasma malondialdehyde (MDA) content of $HgCl_2$ induced oxidative stressed rats. Concentration of MDA is expressed as nmol/ml of plasma. Values are presented as means ± SD. ++P<0.01 compared with oxidative stressed group vs. control. **P<0.01 compared with Hg+ WG vs. oxidative stressed group. (NC = Normal control, Hg = Mercuric chloride, WG = Wheatgrass)

$HgCl_2$ causes the generation of free radicals, reactive oxygen species (ROS) are highly responsible for the lipid peroxidation of biological membranes, which involves decomposition of fatty acids especially (PUFA), production of 4-hydroxynonenal (4HNE) and malondialdehyde (MDA). Lipid oxidation products, such as MDA reacts with proteins, DNA, phospholipids modify RNA, and other biomolecules, another product 4HNE, is hepatotoxic, cytotoxic, genotoxic, mutagenic and induce dysfunction of immune systems. MDA are mostly used parameter for the estimation of the lipid peroxidation by reactive oxygen species (ROS).[56] The result of the study demonstrated that $HgCl_2$ administrated group significantly ($P < 0.01$) enhance in MDA level in comparison with the control group. After wheatgrass treatment MDA level was significantly ($P < 0.01$) decrease in Hg+WG group when compare with $HgCl_2$ treated group[57] Figure 4.

Plasma membrane redox system (PMRS) of Red blood cells (RBCs) transfers electrons from intracellular substrates to extracellular electron acceptors[40] neutralization of oxidative stress, recycling of ascorbic acid and regulating of normal energy metabolism.[58-60] It has been observing in various clinical studies that elevated level of PMRS was found in the erythrocytes during oxidative stress and associated condition such as diabetic nephropathy, type 2 diabetes mellitus and during aging in humans.[61] The result of the present study reveled that $HgCl_2$ administration significantly ($P < 0.001$) increased level of erythrocyte PMRS in $HgCl_2$ treated rats when compare to control group. However, after wheatgrass supplementation the PMRS activity was significantly decreased in Hg+WG treated group as compare to $HgCl_2$-treated group ($P < 0.001$) Figure 5. Elevated level in erythrocyte PMRS activity in $HgCl_2$ treated rats is the indication of excess production of free radicals. Wheatgrass supplementation restored PMRS activity near to normal level, through the scavenging of free radicals and improving the antioxidant system.[55]

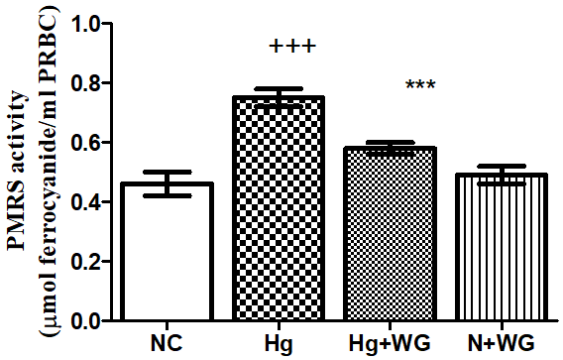

Fig. 5: Effect of wheatgrass treatment on PMRS activity of $HgCl_2$ induced oxidative stressed rats. Concentration of PMRS is expressed as µmol ferrocyanide/ml PRCB. Values are presented as means ± SD. +++P<0.001 compared with oxidative stressed group vs. control. ***P<0.001 compared with Hg+ WG vs. oxidative stressed group. (NC = Normal control, Hg = Mercuric chloride, WG = Wheatgrass)

AOPPs are the biomarkers to estimate the degree of oxidative modifications of proteins.[32] Various pathological conditions such as oxidative stress, hepatotoxicity, kidney disease, diabetes, mental retardation, and muscular dystrophy are associated with excess accumulation of the protein products (AOPPs).[62, 63] In this study researcher observed a significantly ($P < 0.001$) increased formation of AOPP and protein oxidation in mercuric chloride administered rats as compared to control rats. Present study reveled that after the wheatgrass extract supplementation protein oxidation level was significantly decreased ($P < 0.001$) in Hg+WG rats in comparison with $HgCl_2$-treated rats, Figure 6. Therefore, it could be conclude that wheatgrass demonstrated considerable antioxidant efficacy against protein oxidation influence by oxidative stress.

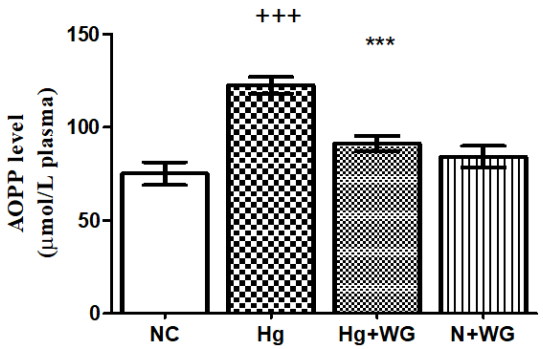

Fig. 6: Effect of wheatgrass treatment on AOPP level of $HgCl_2$ induced oxidative stressed rats. Concentration of AOPP level is expressed as µmol/l of plasma. Values are presented as means ± SD. +++P<0.001 compared with oxidative stressed group vs. control. ***P<0.001 compared with Hg+ WG vs. oxidative stressed group. (NC = Normal control, Hg = Mercuric chloride, WG = Wheatgrass)

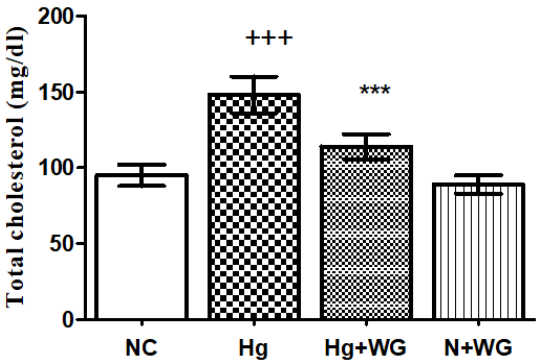

Fig. 7: Effect of wheatgrass treatment on total cholesterol level of $HgCl_2$ induced oxidative stressed rats. Concentration of total cholesterol is expressed as mg/dl. Values are presented as means ± SD. +++P<0.001 compared with oxidative stressed group vs. control. ***P<0.001 compared with Hg+ WG vs. oxidative stressed group. (NC = Normal control, Hg = Mercuric chloride, WG = Wheatgrass)

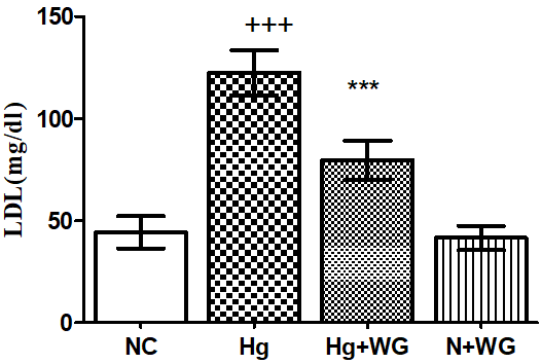

Fig. 8: Effect of wheatgrass treatment on LDL level of $HgCl_2$ induced oxidative stressed rats. Concentration of LDL is expressed as mg/dl. Values are presented as means ± SD. +++P<0.001 compared with oxidative stressed group vs. control. ***P<0.001 compared with Hg+ WG vs. oxidative stressed group. (NC = Normal control, Hg = Mercuric chloride, WG = Wheatgrass

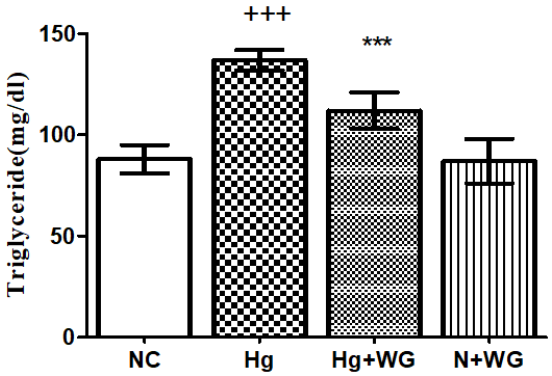

Fig. 9: Effect of wheatgrass treatment on triglyceride level of $HgCl_2$ induced oxidative stressed rats. Concentration of triglyceride level is expressed as mg/dl. Values are presented as means ± SD. +++P<0.001 compared with oxidative stressed group vs. control. ***P<0.001 compared with Hg+ WG vs. oxidative stressed group. (NC = Normal control, Hg = Mercuric chloride, WG = Wheatgrass

Oxidative stress is generally associated with increased formation of free radicals, responsible for the lipid peroxidation; lipid peroxidation makes possible occurrence of cardiac necrosis and accrual of lipids, which leads to injure of cardiac tissues. Cardiac tissue damage induced by $HgCl_2$ in rats was indicated by elevated level of the lipid profile. Elevated level of lipid profile (plasma triglycerides, total cholesterol, LDL-S) in the $HgCl_2$ treated group demonstrated that $HgCl_2$ may be interfering with metabolism or biosynthesis of lipids.[64,65]

Results showed that $HgCl_2$ caused adverse alterations in lipid profiles. Mercuric chloride significantly increased ($p < 0.001$) cholesterol, LDL and triglycerides levels in $HgCl_2$ treated rats as compared to control group. Administration of wheatgrass extract has significantly reduced ($p < 0.001$) elevated TC, LDL and triglycerides levels in Hg+WG treated group as comparison to $HgCl_2$-treated group, Figure 7,8,9. These findings are in parallel with the other experimental studies.[66,67] These changes in lipid profile after wheatgrass treatment may be the presence of its bioactive compounds, flavonoids triterpenoids and tannins, which are reported to lipid lowering effect in many more scientific studies. Wheatgrass supplementation was proficient to condense the $HgCl_2$ induced cardiotoxicity, shows by various ways. Gamma sitosterol an active component of wheatgrass has been reported to persuade cholesterol synthesis in liver and intestine.[54] Studies reveled that anti-platelet aggregation was shown by caryophyllene and its oxides, are active biological components of wheatgrass.[68] Another wheatgrass compounds alpha and beta amyrins are the two biologically active penta cyclic triterpenes, have anti-hyperglycemic effect and hypolipidemic effect and suggesting that this compound is a potential candidate for diabetes and atherosclerosis.[69] This proves that wheatgrass is very efficient in lowering the lipid levels in rats. To conclude, the present result suggests that wheatgrass extract can be used as a lipid lowering agent and as a primary therapy in treating cardiovascular diseases.

Serum urea and creatinine tests are basic parameters of kidney dysfunctions test.[14] By the previous studies it was proved that that kidneys functions are the badly altered by the induction of $HgCl_2$, because Hg accumulates more in kidneys.[70] In the present study, enhanced levels of serum creatinine and urea in $HgCl_2$-treated group indicate the renal dysfunction and nephrotoxicity.[71] This elevation in serum creatinine and urea might be due to damage in renal tissues and alteration in renal functions. Similar findings have stated by the other previous studies.[10,15,72,73] The results demonstrate that increased serum urea and creatinine level was observed in $HgCl_2$- induced stressed rats in comparison with the control rats, ($p < 0.001$), ($p < 0.01$) respectively. On the other hand, treatment with wheatgrass extract at dose 100 mg/kg body weight showed a significant reduction ($p < 0.05$) in

the levels of serum urea and creatinine in Hg+WG treated group as compared to the $HgCl_2$-treated group[14] Figure 10,11. Findings of the study showed that wheatgrass extract improved $HgCl_2$-induced nephrotoxicity; this is due to the antioxidant potential of wheatgrass to quenching free radicals. The antioxidant attributes of wheatgrass was proved by the previous studies, which showed that wheatgrass has a great efficiency for scavenging of ROS as metal chelating agent, and inhibits lipid peroxidation.[15] The antioxidant efficacies of aqueous extract of wheatgrass and suggest that it may be used as a therapeutic agent in the prevention of renal toxicity caused by Hg.[74]

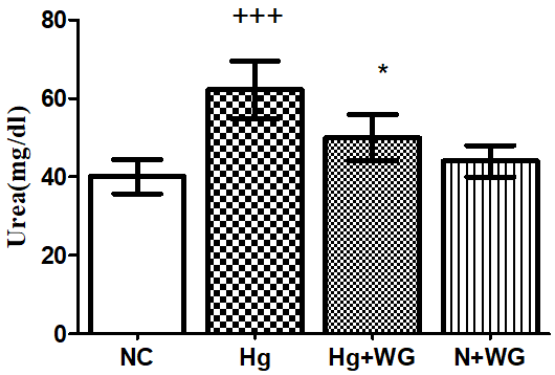

Fig. 10: Effect of wheatgrass treatment on urea level of $HgCl_2$ induced oxidative stressed rats. Concentration of urea is expressed as mg/dl of serum. Values are presented as means ± SD. +++P<0.001 compared with oxidative stressed group vs. control. *P<0.05 compared with Hg+ WG vs. oxidative stressed group. (NC = Normal control, Hg = Mercuric chloride, WG = Wheatgrass)

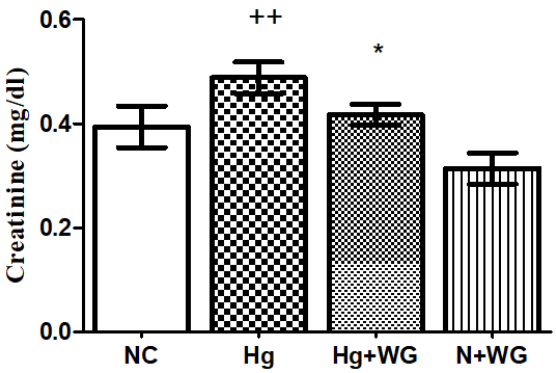

Fig. 11: Effect of wheatgrass treatment on serum creatinine level of HgCl2 induced oxidative stressed rats. Concentration of creatinine is expressed as mg/dl of serum. Values are presented as means ± SD. ++P<0.01 compared with oxidative stressed group vs. control. *P<0.05 compared with Hg+ WG vs. oxidative stressed group. (NC = Normal control, Hg = Mercuric chloride, WG = Wheatgrass

ALP, AST and ALT are the enzymes produced by the liver and all body tissues including muscles, heart, kidney and brain with higher amounts in the liver. Raised levels of these enzymes are the reasonably sensitive indicators of liver damage and tissue injury, where damaged liver cells liberate these enzymes in to the blood circulation and their levels get increased. Elevated levels of these enzymes in stressed conditions, is the initial identification of oxidative stress induced by mercury, which supports the results of this study, our results demonstrated that, liver enzymes (ALP, AST and ALT) levels were augmented in the stressed induced group that confirms occurrence of hepatotoxicity.[75] Therefore

it is concluded that Hg causes hepatotoxicity.[10,76] To estimate the efficacy of wheatgrass extract on $HgCl_2$ -induced hepatic toxicity, liver function tests were measured after wheatgrass supplementation in the serum of $HgCl_2$ treated rats. The finding of the study demonstrated that, $HgCl_2$ administration caused increases ($p < 0.001$) in liver enzymes; ALP, AST and ALT levels, when compare to control rats, while after the supplementation with wheatgrass extract remarkably inhibited $HgCl_2$ -induced liver damage, improved liver functions in Hg+WG treated group when compare to $HgCl_2$-treated group as evidenced by significantly decreased activities of liver enzymes and restored the almost normal serum ALP, AST and ALT levels ($p < 0.001$), ($p < 0.05$), and ($p < 0.001$) respectively, Figure 12,13,14. Findings of the study were in agreement with previous studies, demonstrated hepatoprotective potential of wheatgrass.[77, 78] Wheatgrass and its phytochemicals such as flavonoids, phenols, phytol, sitosterol, squalene, especially alpha and beta amyrins (penta cyclic triterpenes) might be responsible for hepatoprotective potential that improves liver function activities.[79, 80]

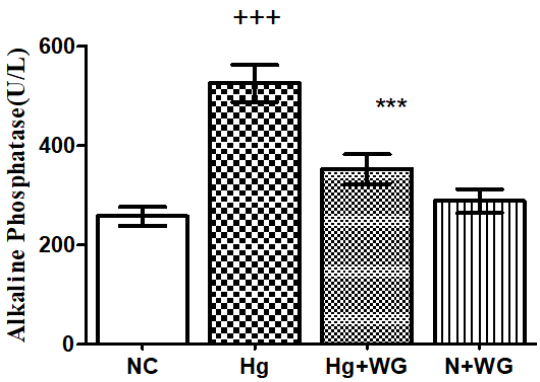

Fig. 12: Effect of wheatgrass treatment on alkaline phosphatase level of $HgCl_2$ induced oxidative stressed rats. Concentration of alkaline phosphatase is expressed as U/L of serum. Values are presented as means ± SD. +++P<0.001 compared with oxidative stressed group vs. control. ***P<0.001 compared with Hg+ WG vs. oxidative stressed group. (NC = Normal control, Hg = Mercuric chloride, WG = Wheatgrass)

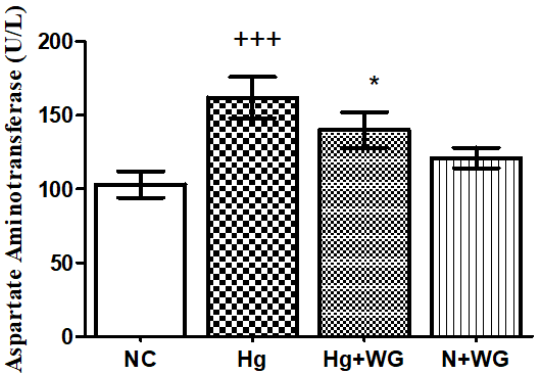

Fig. 13: Effect of wheatgrass treatment on AST level of $HgCl_2$ induced oxidative stressed rats. Concentration of AST is expressed as U/L of serum. Values are presented as means ± SD. +++P<0.001 compared with oxidative stressed group vs. control. *P<0.05 compared with Hg+ WG vs. oxidative stressed group. (NC = Normal control, Hg = Mercuric chloride, WG = Wheatgrass)

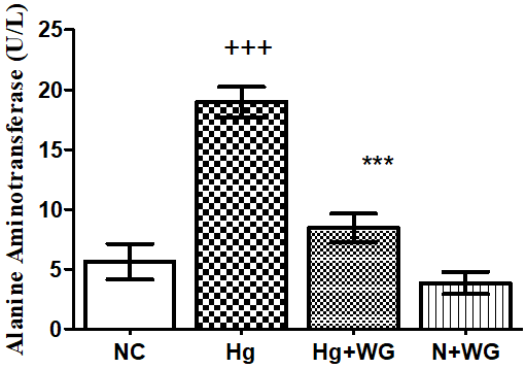

Fig. 14: Effect of wheatgrass treatment on ALT level of $HgCl_2$ induced oxidative stressed rats. Concentration of ALT level is expressed as U/L of serum. Values are presented as means ± SD. +++P<0.001 compared with oxidative stressed group vs. control. ***P<0.001 compared with Hg+ WG vs. oxidative stressed group. (NC = Normal control, Hg = Mercuric chloride, WG = Wheatgrass)

Conclusion

The results of the present study was concludes that wheatgrass act as an efficient antioxidant agent which was confirmed by the findings of the study. Administration of $HgCl_2$ caused oxidative stress was proved by the elevated levels of PMRS, MDA, AOPP, ALP, AST, ALT serum urea, creatinine, TC, TG, LDL simultaneously treatment with wheatgrass significantly improved the antioxidant status-DPPH, FRAP, GHS, hence wheatgrass extract has great potential to improve the antioxidant status and provide protection against mercuric chloride induced oxidative stress and associated complications.

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Conflict of Interest

No conflicts of interest to disclose.

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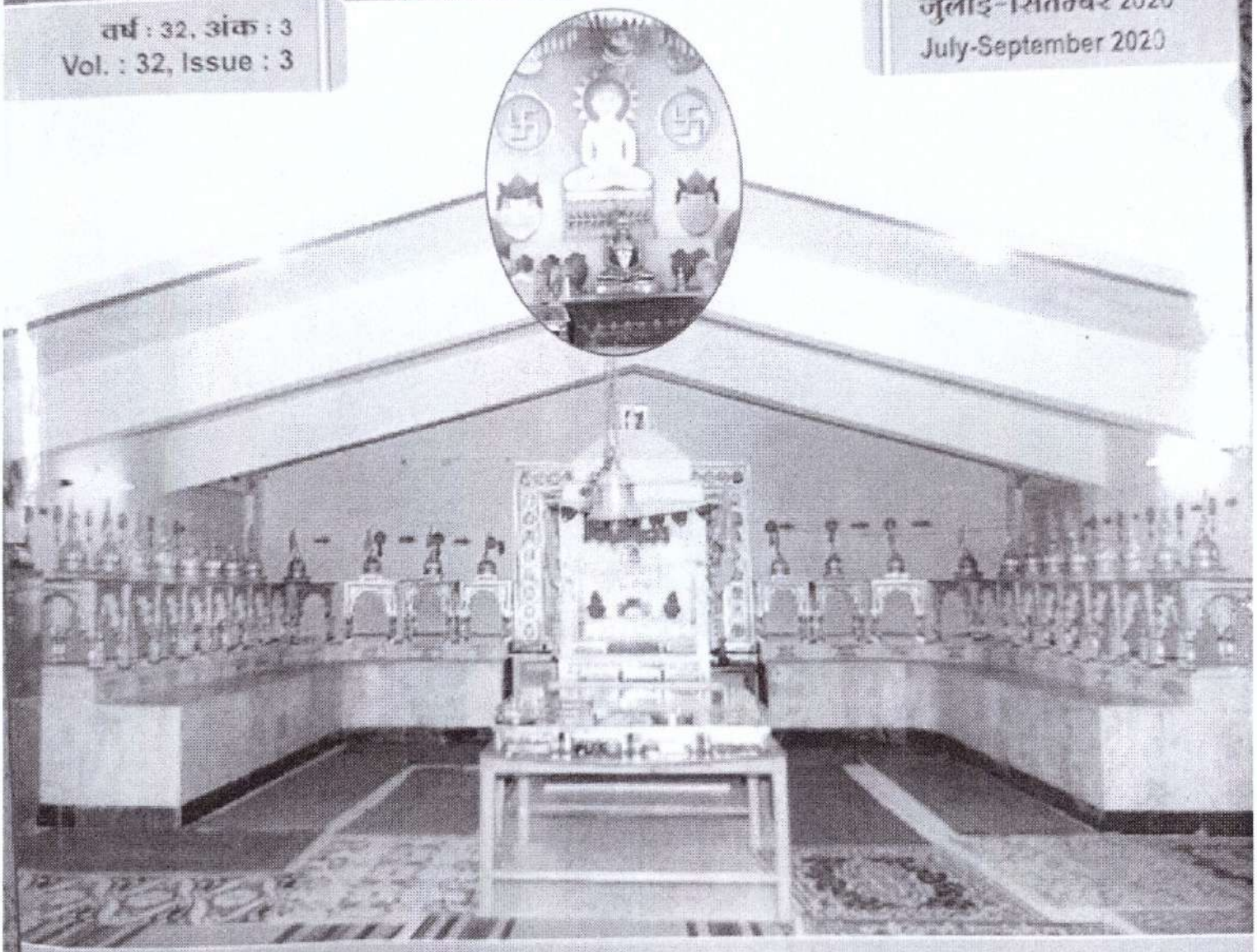

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Fermat Numbers: Need to rename it□

■ Omkar Lal Shrivastava*
■ Sumita Shrivastava**

Abstract

In this paper, we will discuss in particular numbers of the form $2^{2^n}+1$ where n is a Nonnegative integer. These numbers were studied first time in History of Mathematics by Jaina Ācārya Nemicandra but they are called Fermat numbers, named after the French mathematician Pierre de Fermat (1601–1665 A.D.), who studied numbers in this form. Nemicandra Siddhāntacakravartī (981 A.D.) was a great Jaina scholar of Sravanabelgola, Karnataka. Nemicandra Siddhāntacakravartī wrote four canons in Prakṛt namely(i) the Gommaṭasāra Jīvakāṇḍa (734 verses) (ii) the Gommaṭasāra Karmakāṇḍa (972 verses) (iii) the Labdhisāra (with the Kṣapaṇāsāra) (649 verses) (iv) the Tiloyasāra (1018 verses). The Tiloyasāra (Trilokasāra in Sanskrit TLS) of Nemicandra Siddhāntacakravartī is the celebrated work on Karaṇānuyoga. We find Fermat numbers in Tiloyasāra. It is remarkable here that Nemicandra Siddhāntacakravartī knew about Fermat numbers many centuries before Fermat. So it should be renamed as Nemichandra–Fermat Numbers.

Introduction:

1. Fermat's Numbers: We will discuss in particular numbers of the form $2^{2^n}+1$ where n is a nonnegative integer. These are called Fermat numbers, named after the French Mathematician Pierre de Fermat (1601–1665) who first studied numbers in this form. The first seven Fermat's numbers are given below:

$$F_0 = 2^{2^0}+1 = 2^1+1 = 3$$
$$F_1 = 2^{2^1}+1 = 2^2+1 = 5$$
$$F_2 = 2^{2^2}+1 = 2^4+1 = 17$$
$$F_3 = 2^{2^3}+1 = 2^8+1 = 257$$
$$F_4 = 2^{2^4}+1 = 2^{16}+1 = 65537$$

$$F_6 = 2^{2^6}+1 = 2^{64}+1 = 18446744073709551617 = 274177 \times 67280421310721$$

2. Survey of Earlier Literature : The Jaina literature is vast having 58 canonical texts apart from numerous subsidiary texts. These texts deal with religion, philosophy, society, mathematics and other scientific subjects. Research on Jaina

□ It is revised version of invited talk delivered in International Conference of I.S.H.M. organised by M.B.I.T. New Vallabhvidyanagar (Gujarat)

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CERTAIN QUADRUPLE SERIES EQUATIONS INVOLVING LAGUERRE POLYNOMIALS

By

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Abstract

Srivastava ([13], [15]) has solved dual series equations involving Bateman-k functions and Jacobi polynomial Srivastava [16] has obtained more results for the Konhauser-biorthogonal set. Lowndes ([3], [4]), Srivastava [12 Lowndes and Srivastava [5], Srivastava[14], Srivastava and Panda [17] have obtained the solution of dual serie equations involving Jacobi and Laguerre polynomials and also solved triple series equations involving Laguer polynomials. Singh, Rokne and Dhaliwal [10] have find out the solution of triple series equations involving Laguer polynomials in a closed form. Kuldeep Narain ([7], [8]), Rajnesh Krishnan Mudaliar and Kuldeep Narain [6 have solved Certain dual and quadruple series equations involving generalized Laguerre polynomials and Jacol polynomials as kernels. In the present paper, an exact solution has been obtained for the quadruple series equation involving Laguerre polynomials by Noble [9] modified multiplying factor technique.

2010 Mathematics Subject Classifications: 45XX, 33C45, 33D45, 34BXX

Keywords and phrases: Laguerre polynomials, Basic orthogonal polynomials and functions, Boundary valu problems.

1 Introduction

Earlier Srivastava ([13], [15]) has solved dual series equations involving Bateman-k functions and Jacobi polyn Srivastava [16] has obtained more results like generating functions, bilinear generating functions, recurrence re some expansions of functions for the Konhauser-biorthogonal set and general result for the dual series eq involving generalized Laguerre polynomials by putting $k = 1$ in (3.10) and (3.11) in [15,p.645]. Lownd [4]), Srivastava [12], Lowndes and Srivastava [5], Srivastava[14], Srivastava and Panda [17] have obtained the s of dual series equations involving Jacobi and Laguerre polynomials and also solved triple series equations in Laguerre polynomials. Singh, Rokne and Dhaliwal [10] have find out the solution of triple series equations in Laguerre polynomials in a closed form. Kuldeep Narain ([7], [8]), Rajnesh Krishnan Mudaliar and Kuldeep [6] have solved Certain dual and quadruple series equations involving generalized Laguerre polynomials and polynomials as kernels. In this paper, we have obtained the solution of the following quadruple series equation

(1.1) $\sum_{n=0}^{\infty} \frac{A_n}{\Gamma(\alpha+n+p+1)} L_{n+p}^{\alpha}(x) = \phi_1(x), 0 \le x < a,$

(1.2) $\sum_{n=0}^{\infty} \frac{A_n}{\Gamma(\alpha+\beta+n+p)} L_{n+p}^{\alpha}(x) = \phi_2(x), a < x < b,$

(1.3) $\sum_{n=0}^{\infty} \frac{A_n}{\Gamma(\alpha+n+p+1)} L_{n+p}^{\alpha}(x) = \phi_3(x), b < x < c,$

(1.4) $\sum_{n=0}^{\infty} \frac{A_n}{\Gamma(\alpha+\beta+n+p)} L_{n+p}^{\alpha}(x) = \phi_4(x), c < x < \infty,$

where $0 < \beta + m$, $0 < \alpha + \beta < \alpha + 1$, p and m are non-negative integer.

(1.5) $L_{n+p}^{\alpha}(x) = \binom{\alpha+n+p}{n+p} F_1[-n-p, \alpha+1, x]$

is the Laguerre Polynomial, $\phi_1(x), \phi_2(x), \phi_3$ and $\phi_4(x)$ are prescribed functions.

The solution presented in this paper is obtained by employing a multiplying factor technique similar to th by Noble [9] or Lowndes ([3],[4]).

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The Antioxidant Efficacy of Wheatgrass (*Triticum aestivum*) on Mercuric Chloride ($HgCl_2$) -Induced Oxidative Stress in Rat Model

RENU TRIPATHI[1], SWATI AGARWAL[2], SYED IBRAHIM RIZVI[3] and NEETU MISHRA[4*]

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Abstract

Mercury is a harmful toxic pollutant, which has hepato-nephrotoxic, hematotoxic, genotoxic and neurotoxic effects. The aim of the study was to evaluate the protective efficacy of wheatgrass on mercuric chloride ($HgCl_2$) induced oxidative stress and associated complications in rat model. Albino rats were divided into four groups (three rats per group). Group I normal control group. Group II oxidative stressed group received mercuric chloride (0.5 mg/kg/day). Group III only received wheatgrass extract (100 mg/kg/day), whereas Group IV received wheatgrass (100 mg/kg/day) after one hour, followed by mercuric chloride (0.5 mg/kg/day) for 30 days. The results of the study showed that wheatgrass supplementation significantly decreased the $HgCl_2$ induced elevated oxidative stress parameters Plasma Malondialdehyde (MDA) content, Plasma membrane redox system (PMRS), Advanced oxidation protein products (AOPP), simultaneously elevated lipid profile (Total Cholesterol, Triglycerides, Low-density lipoprotein (LDL), liver enzymes as, Plasma Alkaline phosphatase (ALP), Aspartate aminotransferase (AST), and Alanine aminotransferase (ALT), Serum Urea, and Creatinine levels in rats. In addition, wheatgrass treatment improved the antioxidant status in terms of intracellular Reduced Glutathione (GSH), Ferric reducing antioxidant power (FRAP) and 2, 2- diphenyl -1- picrylhydrazyl (DPPH). Therefore it can be concluded that wheatgrass has great potential to diminish the stress-mediated complications and improve the antioxidant status.

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Keywords

Antioxidant; Hepato-Protective; Mercuric Chloride; Nephro-Protective; Oxidative Stress; Wheatgrass.

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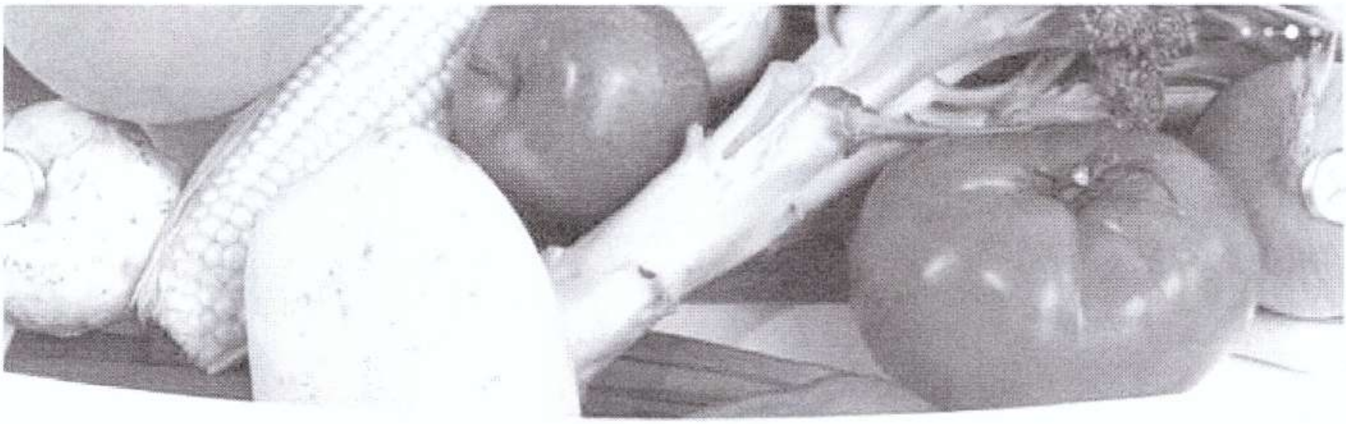

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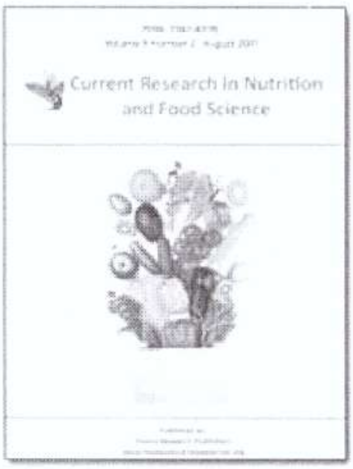

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The Antioxidant Efficacy of Wheatgrass (Triticum Aestivum) on Mercuric Chloride (HgCl2) - Induced Oxidative Stress in Rat Model

Pages : 450-464

Renu Tripathi[1], Swati Agarwal[2], Syed Ibrahim Rizvi[3] and Neetu Mishra[4*]

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Investigating The Bioactive Properties of Cheese-Fruit Combinations Following In Vitro Digestion Using an Elderly Model.

Pages : 465-478

Aimee M. Plante[1*], Aoife L. McCarthy[1], Seán Lacey[2] and Fiona O'Halloran[1]

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Effects of Chewing Gum Stimuli on General and Emotional Stroop Test

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Sung-Yoon WON[1] and Dong-Eun LEE[2*]

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Antifungal Activity of Carboxymethyl Cellulose Edible Films Enriched with Propolis Extracts and Their Role in Improvement of the Storage Life of Kashkaval Cheese

Pages : 487-499

Yulian Dimitrov Tumbarski[1*], Mina Mihaylova Todorova[2], Mariyana Georgieva Topuzova[2], Petya Ivanova Georgieva[3], Zlatka Angelova Ganeva[1],

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EFFECTS OF THYMOL SUPPLEMENTATION AGAINST TYPE 2 DIABETES IN STREPTOZOTOCIN-INDUCED RAT MODEL

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Abstract

The present investigation was aimed to study the effect of thymol, an active compound of *Nigella sativa*, on streptozotocin (STZ) induced diabetic rats. Thirty male Wistar rats were randomly divided into five groups (i) Normal control, (ii) Diabetic control, (iii) Diabetic treated with insulin (iv) Diabetic treated with thymol and (v) Normal rats treated with thymol. The rats were injected with STZ at a dose of 55 mg/kg body weight intraperitoneally to induce diabetes. Thymol was given orally at a dose of 40mg/kg body weight for 28 days. At the end of the study, the rats were sacrificed and blood samples were drawn by cardiac puncture to determine the levels of Blood glucose, Plasma Alkaline phosphatase (ALP), Total Cholesterol, Triglycerides, Low-density lipoprotein (LDL), High-density lipoprotein (HDL), Urea, Creatinine, Serum glutamic-oxaloacetic transaminase (SGOT), Serum glutamate pyruvate transaminase (SGPT), Plasma malondialdehyde (MDA) content, Advanced oxidation protein products (AOPP), Sialic acid (SA). Markers of antioxidants as Ferric reducing antioxidant power (FRAP) and erythrocyte Reduced Glutathione (GSH) were also measured. Radical scavenging activity in terms of percent 2, 2-diphenyl -1- picrylhydrazyl (DPPH). Blood glucose level, Total Cholesterol, Triglycerides, LDL, Urea, Creatinine, SGOT, SGPT, ALP, MDA, AOPP, SA levels which were found elevated due to diabetes reduced significantly to near normal. However, HDL level, % DPPH, FRAP content and erythrocyte GSH levels elevated after supplementation. We observe that administration of thymol afforded remarkable protection against diabetes and its related complications.

Key words: Thymol, *Nigella sativa*, Streptozotocin, Cholesterol.

Introduction

Diabetes mellitus is one of the most common disorders affecting almost 8.8% of the world's population. According to International Diabetes Federation (IDF) estimates there are 424.9 million adults (aged 20-79) worldwide with diabetes mellitus (DM), a number that is expected to reach to 628.6 million by 2045 (IDF, 2017).

Globally, diabetes is one of the six major causes of death (Petchi *et al.*, 2014). Medical interventions to manage diabetes include glucose-lowering agents such as alpha-glucosidase inhibitors, biguanides, sulfonylureas and thiazolidinediones and sometimes insulin. All these interventions are associated with severe to mild side effects after a given amount of time. These compulsions provide a perfect setting for use of alternative systems of medicine based on traditional knowledge (Parasuraman *et al.*, 2010).

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Plants and their active compounds have frequently been used for medicinal purposes. According to the World Health Organization (WHO), "a medicinal plant is a plant which, in one or more of its organs, contains substances that can be used for therapeutic purposes, or which are precursors for chemo-pharmaceutical semi-synthesis." Such plants are in great demand by pharmaceutical companies for their active ingredients (Huai *et al.*, 2010, Husain *et al.*, 2008).

In the Indian traditional system of medicine, *Nigella sativa*, an annual herb belonging to Ranunculaceae family have been used for thousands of years as a spice and food preservative to a variety of food products as bread, yogurt, pickles, sauces, salads etc. (Hajhashemi *et al.*, 2004). *Nigella sativa* is traditionally used for its galactagogue, appetizer, thermogenic and diuretic effects (Hosseinzadeh *et al.*, 2013). Furthermore, *Nigella sativa* possesses anti-microbial, anti-fungal, anti-oxidative and

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AN INTERNATIONAL BILINGUAL PEER REVIEWED REFEREED RESEARCH JOURNAL

A STUDY OF SATISFACTION LEVEL OF FARMERS FROM CO-OPERATIVE SOCIETY (WITH SPECIAL REFERENCE TO TRIBAL BLOCK DONDI IN BALOD DISTRICT,C.G.)

Dr. Dharmender Singh*
Dr. Lalee Sharma**

ABSTRACT

The co-operative movement has been necessitated to protect the interests of weaker sections of society. The primary objective of this movement is ***'how to protect economically weaker sections of society'****. Chhattisgarh cooperative marketing federation (MARKFED) is an important institution which procures agriculture products from farmers and distributes chemical fertilizers to farmers through PACS on online platform. MARKFED is an agency of government to procure paddy and distribute fertilizers and pesticides through PACS on online platform. PACS works as a mediator between MARKFED and farmers. PACS directly deals with farmers for procurement of paddy and distribution of fertilizers. Present study is carried on to know about satisfaction level of farmers with procedure of paddy procurement and distribution.*

Keywords *- PACS, Co-operative society, MARKFED, Paddy,Procurement, Fertilizer*

INTRODUCTION

The cooperatives sector has made significant contributions in agro-processing, distribution of inputs like fertilizers and pesticides, storage and marketing. Marketing cooperatives deals with the marketing of agricultural production such as food grains and commercial crops. Marketing cooperative also helps to meet the requirements of the farmers. The Cooperative marketing has conferred multifarious advantages on the farmers. Now, instead of marketing their produce separately; they market it together through one agency. Chhattisgarh cooperative marketing federation (MARKFED) is an important institution which procures agriculture products from farmers and distributes chemical fertilizers to farmers through PACS on online platform.

In this research considerable endeavor have put to understand the satisfaction from working procedure of PACS, concept of the principle ofcooperation, genesis of cooperative movement in tribal block Dondi. Here again in literature considerable focus was given to understand the roles and functions of the cooperative society and the problems mounting of overdue faced by these societies and their subsequent failures. Based on the understanding of the subject matter the research objectives and methodology to study the cooperative society was framed in consultation with the faculty guide.

Objective of Study

1. To know about the position of the Cooperative society in Dondi block.
2. To know the different works done by the societies.

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AN INTERNATIONAL BILINGUAL PEER REVIEWED REFEREED RESEARCH JOURNAL

A STUDY OF SATISFACTION LEVEL FROM BANKING FACILITY PROVIDED BY WOMEN COOPERATIVE BANK TO ITS MEMBERS.

Dr. Dharmender Singh*
Dr. Lalee Sharma**

ABSTRACT

With the increasing role of women in family matters, it has become necessary that they become partners in the economic matters of the family as well. Women should also be aware of all the means of banking and savings that they do not have. Women Cooperative Bank has been a pioneer in discharging social responsibilities from time to time, financial and social upliftment of women, saving spirit, help of Naxal victims and health fair have always been in the forefront. Pragati Mahila Nagarik Sahakari Bank's function commenced 20 years ago from Maharashtra Mandal Sector 4, Bhilai. Study was based on satisfaction and privileged of member women. The statistical study and analysis revealed that this bank is fulfilling the needs of financial inclusion of members and they are satisfied with banking facility provided.

Keywords:- *Women Empowerment, financial inclusion, women cooperative banks.*

1. Introduction: -

1.1 Empowerment of women through co-operative

General and commercial bankers have considered the poor to be non-bankable customers, so the President of the Working Women Forum to help the women, especially the poor women, has organized a successful co-operative organization naming The Indian co-operative Network for Women in the southern Indian states of Tamilnadu, Andhra Pradesh and Karnataka with co-operation of highly poor women workers to help the asset less and financially weaker women and to benefit the poor and improve their quality of life. WWF and Indian co-operative network for women both have jointly promoted the unique framework from the beginning to reach out to a large number of poor personnel.

Since there was a provision to improve the standard of living of millions of people and women under the cooperative movement, only cooperative enterprises were considered suitable by the founding members to remove all forms of discrimination among the weaker sections.

Objective:-

The major objectives of the research study are:

1. To study satisfaction level from facilities provided by women cooperative banks to women.
2. To study the satisfaction level of women members of the bank towards women cooperative banks.
3. To study the satisfaction level of bank women members through the working of women Co-operative Bank.

2. Hypothesis:-

The following hypotheses have been formulated with reference to the problem identified: -

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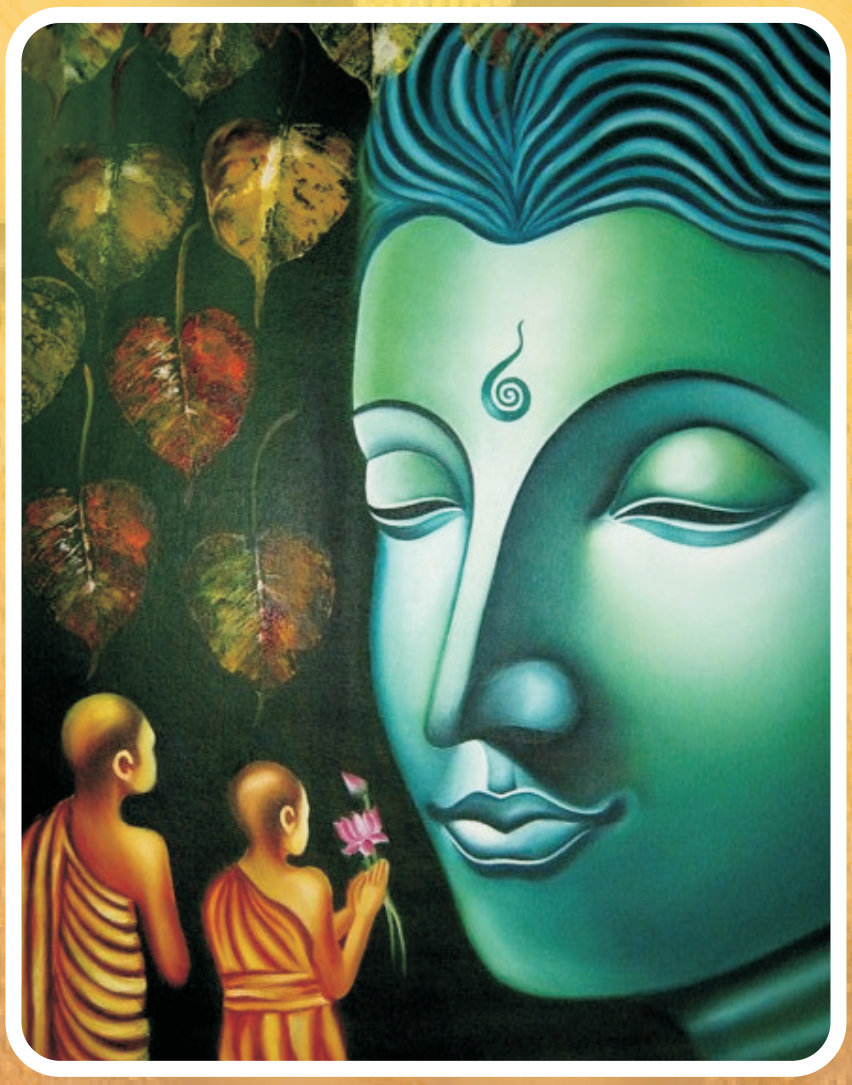

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अनुक्रमणिका

इस अधिनियम के तहत पुरूषों व महिलाओं के बीच किसी भी भेदभाव की अनुमति नही है इसलिए पुरूषों व महिलाओं को समान वेतन भुगतान किया जाना चाहिए सभी वयस्क रोजगार हेतु आवेदन कर सकते है।

छ.ग. में मनरेगा योजना का प्रारंभ :–

महात्मा गांधी राष्ट्रीय रोजगार गारंटी अधिनियम 2005 दिनांक 07 सितम्बर 2005 को जारी किया गया। महात्मा गांधी राष्ट्रीय रोजगार गारंटी अधिनियम 2005 की धारा 4(1) अंतर्गत छत्तीसगढ़ राज्य में 2 फरवरी 2006 से मनरेगा योजना प्रारंभ की गई।

प्रथम चरण मे दिनांक 02 फरवरी 2006 से राज्य के 11 जिले बस्तर, बिलासपुर, दंतेवाड़ा, धमतरी, जशपुर, कांकेर, कबीरधाम, कोरिया, रायगढ़, राजनांदगाव एवं सरगुजा में व द्वितीय चरण में दिनांक 1 अप्रैल 2007 से चार जिले रायपुर, जांजगीर–चांपा, कोरबा व महासमुंद में तथा तृतीय चरण में दिनांक 1 अप्रैल 2008 से समस्त जिलों में योजना लागू की गई।

मनरेगा योजना के प्रमुख उद्देश्य :–

1. अधिनियम के अंतर्गत ग्रामीण क्षेत्र में परिवारों को (एक वित्तीय वर्ष में 100 दिवस का) वयस्क सदस्यों को अकुशल मानव काम रोजगार सुनिश्चित करना एवं स्थायी परिसंपत्तियों का सृजन करना।
2. छ.ग. में मनरेगा के अंतर्गत 100 दिवस से बढ़ाकर 150 दिवस रोजगार प्रदान किया जा रहा है। अतिरिक्त 50 दिवस रोजगार उपलब्ध पर होने वालों व्यय राज्य शासन द्वारा वहनीय है।
3. किसी भी ग्रामीण परिवार के वयस्क सदस्य जो कुशल शारीरिक श्रम करने को तैयार है। उनके द्वारा आवेदन किये जाने के 15 दिवस के भीतर रोजगार उपलब्ध कराने की गारंटी लागू होती है।
4. आवेदक को 15 दिवस के भीतर कार्य उपलब्ध नही कराने पर बेरोजगारी भत्ता दिया जाता है। बेरोजगारी भत्ता प्रथम 30 दिवस हेतु न्यूनतम मजदूरी दर का 1/4 होता है एवं 30 दिवस के उपरांत न्यूनतम मजदूरी दर का 1/2 होता है।
5. योजना के अंतर्गत मजदूरी का भुगतान बैंक/डाक पर के बचत खातों के माध्यम से किया जाता है।
6. योजनांतर्गत वर्तमान वित्तीय वर्ष में रू.172 प्रति दिवस मजदूरी दर भारत सरकार द्वारा निर्धारित किया गया है।
7. योजना के अंतर्गत ग्राम पंचायत स्तर पर मजदूरी व सामग्री का 60 रू. 40 के अनुपात में राशि व्यय का प्रावधान है।

अध्ययन के प्रमुख उद्देश्य :–

1. छ.ग. में मनरेगा योजना द्वारा उपलब्ध कराये जा रहे रोजगार के अवसरों का अध्ययन करना।

2. छ.ग. में मनरेगा योजना द्वारा उपलब्ध कराये जा रहे आय के अवसरों का अध्ययन करना।
3. छ.ग. में मनरेगा योजना हेतु उपलब्ध किये जा रहे केन्द्रीय सहायता अध्ययन करना।
4. छ.ग. में मनरेगा योजना अंतर्गत आंबटित राशि के उपयोग व्यय का अध्ययन करना।

अध्ययन का सीमाएँ :–

1. छ.ग. राज्य में संचालित मनरेगा योजना का अध्ययन द्वितीयक आकडो पर आधारित है।
2. मनरेगा योजना के अध्ययन के लिए 5 वर्षो के आँकड़े वर्ष 2013–14 से 2017–18 (फरवरी) तक का प्रयोग किया गया है।
3. मनरेगा योजना के उपलब्ध कराए गए आँकड़े वार्षिक वित्तीय प्रतिवेदन से प्राप्त जानकारी पर ही आधारित है।

छ.ग. में मनरेगा की स्थिति (वित्तीय वर्ष 2017–18 में 5/2/2018 की)

1.	कुल जॉब कार्ड जारी (लाख में)	–	37.37
2.	कुल श्रमिकों की संख्या (लाख में)	–	84.00
3.	कुल सक्रिय जॉब कार्ड (लाख में)	–	29.00
4.	कुल सक्रिय श्रमिकों की संख्या (लाख में)	–	57.12
5.	अनुसूचित जाति के श्रमिकों का प्रतिशत	–	10.58
6.	अनुसूचित जनजाति के श्रमिकों का प्रतिशत	–	34.09

छ.ग. में मनरेगा योजना के अंतर्गत श्रम बजट, कार्य दिवस उत्पन्न, औसत रोजगार उपलब्धता प्रति परिवार, औसत मजदूरी प्रति व्यक्ति प्रति दिवस आदि का विवरण

(राशि लाख रू. में

	वित्तीय वर्ष	वित्तीय वर्ष	वित्तीय वर्ष	वित्तीय वर्ष	वित्तीय वर्ष
विवरण	2017–18	2016–17	2015–16	2014–15	2013–14
अनुमोदित श्रम बजट (लाख में)	1000	900	1234.02	624.39	1214.36
कार्य दिवस उत्पन्न(लाख में)	883.10	885.94	1013.90	555.90	1298.84
कार्य दिवस का प्रतिशत %	88.31	98.44	82.13	89.03	106.96
अनुसूचित जाति के श्रमिकों का कार्य दिवस % प्रतिशत	10.08	9.01	8.38	10.81	9.02
अनुसूचित जनजाति के श्रमिकों का कार्य दिवस का कुल कार्य दिवस पर % प्रतिशत	35.92	38.45	42.55	32.03	40.12
महिला श्रम कार्य दिवस का कुल श्रम पर दिवस % प्रतिशत	49.46	49.31	49.02	49.87	48.53
औसत रोजगार उपलब्धता प्रति परिवार	42.48	41.55	46.64	31.80	51.71
औसत मजदूरी प्रति व्यक्ति प्रति दिवस	165.66	158.98	152.80	150.18	142.98
100 दिवस रोजगार पूर्ण करने वाले परिवार	169090	172904	242579	48087	346287
कुल श्रमिक परिवार (लाख में)	20.79	21.32	21.74	17.48	25.12
कुल व्यक्तिगत श्रमिक	37.27	39.98	41.17	32.59	49.90

सारिणी के निरीक्षण से स्पष्ट रूप से दर्शित हो रहा है कि वर्ष 2013–14 में मनरेगा के अंतर्गत श्रम बजट में अनुमोदित राशि 1214.36 लाख रू है जो कि वर्ष 2017–18 में घटकर 1000 लाख रू .पर पहुंच गया है। वर्ष 2015–16 में अनुमोदित श्रम बजट में कुछ वृद्धि दर्शित हो रही है।

सारणी के निरीक्षण से स्पष्ट हो रहा है कि वर्ष 2013–14 में कुल 1298.84 लाख रू. का कार्य दिवस उत्पन्न हुआ जो कि वर्ष 2017–18 मे घटकर 883.10 लाख रू. कार्य दिवस रह गया है।

सारणी के निरीक्षण से स्पष्ट हो रहा है कि वर्ष 2013–14 में ही अनुमोदित श्रम बजट के 106.96 प्रतिशत कार्य दिवस उत्पन्न किया गया। वर्ष 2016–17 में 98.44 प्रतिशत कार्य दिवस उत्पन्न किया गया। जबकि अन्य वर्षो में अनुमोदित श्रम बजट के अनुरूप कार्य दिवस उत्पन्न करने में उतनी सफलता प्राप्त नही हुई है।

अनुसूचित जनजाति के श्रमिकों के कार्य दिवस के आंकड़ों का निरीक्षण करने से विदित हो रहा है कि वर्ष 2013–14 के कुल कार्य दिवस में अनु जनजाति के श्रमिकों के कार्य दिवस का प्रतिशत 40.12 था वह वर्ष 2017–18 में घटकर 35.92 प्रतिशत रह गया है। वर्श 2015–16 में अनुसूचित जनजाति के श्रमिकों के कार्य दिवसों का कुल कार्य दिवस से प्रतिशत से 42.55 रहा।

वर्ष 2013–14 में अनुसूचित जाति के श्रमिकों के कार्य दिवस का कुल कार्य दिवस से प्रतिशत 9.02 था जो कि वर्ष 2017–18 में बढकर 10.08 प्रतिशत हो गया है।

वर्ष 2013–14 में जहां कुल श्रम दिवस में महिला श्रम दिवस का प्रतिशत 48.53 था वह 2017–18 में

बढ़कर 49.46 प्रतिशत हो गया है। वर्श 2014–15 में कुल श्रम दिवस में महिलाओं की भागीदारी 49.87 प्रतिशत, वर्श 2015–16 में भागीदारी 49.02 तथा वर्श 2016–17 में महिलाओं की भागीदारी 49.31 प्रतिशत रही।

मनरेगा के अंतर्गत वर्ष 2013–14 में जहां औसत रोजगार उपलब्धता प्रति परिवार 51.71 है, वहीं वर्ष 2017–18 में औसत रोजगार उपलब्धता प्रति परिवार 42.48 है। वर्ष 2014–15 में औसत रोजगार उपलब्धता प्रति परिवार 31.80 न्यूनतम रही।

छ.ग. में मनरेगा के अंतर्गत 100 दिवस रोजगार पूर्ण करने वाले परिवारों की संख्या वर्ष 2013–14 से 2017–18 तक की अवधि में, वर्ष 2014–15 को छोड़कर अन्य वर्षो में निरंतर कमी दर्शित हो रही है। वर्ष 2014–15 में 100 दिवस का रोजगार पूर्ण करने वाले परिवारों की संख्या में भारी कमी दर्शित हो रही है। वर्ष 2013–14 में 100 दिवस का रोजगार पूर्ण करने वाले परिवारों की संख्या 346287 थी जो कि वर्ष 2017–18 में से घटकर 169090 रह गई है।

तालिका के निरीक्षण से यह भी विदित हो रहा है कि वर्ष 2013–14 में कुल श्रमिक परिवार 25.12 लाख दर्ज है जिसकी संख्या वर्ष 2014–15 में घटकर 17.48 लाख हो गई, वर्ष 2015–16 में श्रमिक परिवार की संख्या पुनः बढकर 21.74 लाख हो गई, वर्ष 2016–17 में श्रमिक परिवार की संख्या 21.32 लाख एवं वर्ष 2017–18 में यह संख्या घटकर 20.79 लाख रह गई है।

छ.ग. में मनरेगा योजना के अंतर्गत केन्द्रीय सहायता, उपयोग का प्रतिशत,
कुल व्यय एवं कुल कार्य पूर्ण आदि का विवरण्र

विवरण	2017–18 (5/2/2018 तक)	2016–17	2015–16	2014–15	2013–14
कुल केन्द्रीय सहायता	236261.88	223193.55	106341.30	150570.49	144038.81
कुल उपलब्धता	267545.66	266013.14	128388.70	178153.47	219960.87
कुल व्यय (लाख रू में)	264796.41	275271.11	128304.07	175245.03	202364.04
उपयोग का प्रतिशत	98.97	103.48	99.93	98.37	92
मजदूरी(लाख रू में)	150847.49	200859.83	86425.59	124318.14	147203.37
सामग्री व कुषल श्रम (लाख रू में)	105191.54	63852.99	34721.35	40470.08	44019.63
मजदूरी दायित्व (लाख रू में)	3055.78	1163.07	656.95	279.48	370.90
औसत लागत प्रति व्यक्ति प्रति दिवस (रू)	243.78	221.65	178.62	229.58	185.20
कुल व्यय जो Efms से % प्रतिशत	99.94	97.76	96.07	43.54	8.18
15 दिन में मजदूरी भुगतान का % प्रतिशत	94.49	25.11	7.81	28.64	50.5

सारिणी के निरीक्षण से स्पष्ट रूप से दर्शित हो रहा है कि जब वर्ष 2013–14 में मनरेगा के अंतर्गत केन्द्रीय सहायता 144038.81 लाख रू है वो वर्ष 2015–16 में घटकर 106341.30 लाख रू है। वर्ष 2017–18 में केन्द्रीय सहायता 236261.88 लाख रू रही है। जो कि वर्ष 2013–14 की तुलना में में 64.03 प्रतिशत अधिक हो गया है।

उपयोग के प्रतिशत के आंकड़ों का निरीक्षण करने से विदित हो रहा है कि वर्ष 2013–14 में कुल व्यय का कुल उपलब्धता से प्रतिशत 92.00 था वर्ष 2014–15 में यह प्रतिशत 98.37 हो गया, वर्ष 2015–16 में 99. 93 प्रतिशत, वर्ष 2016–17 में प्रतिशत बढकर 103.48 हो गया, वर्ष 2017–18 में 98.97 प्रतिशत रह गया है। वर्ष 2016–17 में उपयोग का प्रतिशत 103.48 सर्वाधिक रहा ।

छ.ग. में मनरेगा के अंतर्गत रोजगार करने वाले श्रमिकों की मजदूरी दायित्व वर्ष 2013–14 में 370.90 लाख रू था जो कि वर्ष 2017–18 में बढकर 3055.78 लाख रू हो गया है। वर्ष 2014–15 में मजदूरी दायित्व 229.58 लाख रू न्युनतम रही है।

मनरेगा के अंतर्गत औसत लागत प्रति व्यक्ति प्रति दिवस वर्ष 2013–14 में 185.2 रू है। वर्ष 2017–18 में बढकर 243.78 रू प्रति व्यक्ति प्रति दिवस हो गइ है। वर्ष 2015–16 में औसत लागत प्रति व्यक्ति प्रति दिवस 178.62 रू न्युनतम रही है। 2017–18 में प्रति व्यक्ति प्रति दिवस औसत लागत 243.78 रू सर्वाधिक है।

छ.ग. में मनरेगा के अंतर्गत रोजगार पूर्ण करने वाले परिवारों को 15 दिन में मजदूरी भुगतान का प्रतिशत वर्ष 2013–14 से 2017–18 तक की अवधि में वर्शवार क्रमशः 50.5 28.64 7.81 25.11 94.49 प्रतिशत रहा । वर्ष 2015–16 में यह प्रतिशत 7.81 न्युनतम रहा है। वर्ष 2017–18 में 15 दिन में मजदूरी भुगतान का प्रतिशत सर्वाधिक 94.49 है।

छ.ग. में मनरेगा योजना के अंतर्गत कुल कार्य लिये गए**,** चालू कार्य एवं कुल कार्य पूर्ण आदि का विवरण

	वित्तीय वर्ष	वित्तीय वर्ष	वित्तीय वर्ष	वित्तीयवर्ष	वित्तीय वर्ष
विवरण	2017–18	2016–17	2015–16	2014–15	2013–14
कुल लिये गए कार्य (लाख में)	165.87	167.87	123.12	97.65	93.52
कुल चालू कार्य	123.35	102.34	86.94	68.21	66.10
कुल कार्य पूर्ण	42.52	65.53	36.18	29.44	27.42

सारिणी के निरीक्षण से स्पष्ट रूप से दर्शित हो रहा है कि वर्ष 2013–14 में मनरेगा के अंतर्गत कुल 93.52 लाख कार्य प्रारंभ किए गए इसमें से 27.42 लाख कार्य पूर्ण हुए है जो कि कुल लिये गए कार्य का 29.32 प्रतिशत था जो कि वर्ष 2017–18 में घटकर 25.63 प्रतिशत हो गया है। वर्ष 2016–17 में सर्वाधिक कार्य पूर्ण हुये जिसकी राशि 65.53 लाख रू तथा पूर्णताः का प्रतिशत 39.04 रहा।

असफलतायेंः–

1. मनरेगा योजना के अंतर्गत छ.ग. में रोजगार उपलब्ध कराने की गति धीमी है। छ.ग. में वित्तीय वर्ष 2016–17 में 9 फरवरी 2017 में मनरेगा परिवारों को जॉब कार्ड जारी किया गया है।

वित्तीय वर्ष	परिवारों को रोजगार 100 दिवस का उपलब्ध कराया
2015–16	11 %
2016–17	5.21 %

वित्तीय वर्ष 2016–17 के दौरान 2350401 परिवारों ने रोजगार की मांग की। इनमें से 1940274 को रोजगार उपलब्ध कराया गया है। इस प्रकार कुल 88.55% परिवारों को रोजगार दिया गया है। लेकिन इनमें से 101278 परिवारों को ही 100 दिवस का रोजगार उपलब्ध कराया गया। जो कि मात्र 5.21% है। रोजगार उपलब्धता की धीमी गति रही तो छ.ग. से न तो पलायन रूकेगा और न हीं कुपोषण मिटेगा।

2. मनरेगा अंतर्गत कभी–कभी केन्द्र सरकार से फंड/कोष प्राप्त नही होने के कारण मजदूरी का भुगतान रोक दिया जाता है। जिससे मजदूरी करने वाले परिवारों के भरण पोषण व अन्य कृषि कार्यो के लिए राशि न होने के कारण मुश्किलों का सामना करना पड़ता है।
3. केन्द्र द्वारा देश भर में छत्तीसगढ़ राज्य में मनरेगा की मजदूरी को सबसे कम निर्धारित की जाती है। रोजगार की कमी व बेरोजगारों की अधिक संख्या के चलते यहां मजदूरों को सबसे सस्ता मान लिया गया है। केन्द्र सरकार द्वारा हरियाणा, चंडीगढ़, पंजाब, केरल, कर्नाटक, गोवा अन्य राज्यों में भी ज्यादा मजदूरी तय की गई है। झारखंड व उड़ीसा से भी कम मजदूरी छ.ग. के लिए निर्धारित की गई है।

केन्द्र सरकार द्वारा निर्धारित की गई मनरेगा की मजदूरी दरें

वित्तीय वर्ष	छ.ग. में मजदूरी	उत्तरप्रदेष में मजदूरी	पंजाब में मजदूरी	तमिलनाडु में मजदूरी
2015–16	159	161	210	163
2016–17	167	174	218	183
2017–18	172	175	233	205

4. मनरेगा ग्रामीण विकास व रोजगार में दोहरे लक्ष्य को प्राप्त करता है। मनरेगा अंतर्गत कार्य को ग्रामीण विकास गतिविधियों के एक विशिष्ट सेट की ओर उन्मुख होना चाहिए जैसे जल संरक्षण व संचयन, वनीकरण, ग्रामीण संपर्क तंत्र, बाढ़ नियंत्रण व सुरक्षा जिसमें तटबंधों का निर्माण व मरम्मत, नए टैंक व तालाबों की खुदाई, रिसाव टैंक व छोटे बांधों के निर्माण को भी महत्व दिया जाता है। कार्यरत् लोगों की

भूमि समतल, वृक्षारोपण जैसे कार्य किये जाते है।

मनरेगा योजना के संचालन की गतिविधियां मानकीकृत है। इसमें स्थानीय परामर्श नही के बराबर था।

मनरेगा के सुचारू संचालन हेतु राज्य शासन द्वारा की गई व्यवस्था/उठाये गये कदम :–

1. भारत सरकार के निर्देशानुसार पारदर्शिता सुनिश्चित करने हेतु राज्य में पृथक से सामाजिक अंकेक्षण इकाई का गठन किया गया है।
2. मनरेगा अंतर्गत प्राप्त शिकायतों के निवारण हेतु ''छत्तीसगढ़ ग्रामीण रोजगार गारंटी शिकायत निवारण नियम 2012'' का राजपत्र में 11 मई 2012 को प्रकाशन किया गया।
3. राज्य व जिला स्तर पर शिकायतों के तत्काल निपटारे हेतु हेल्प लाईन/टोल फ्री नंबर की व्यवस्था की गई।
4. मनरेगा अंतर्गत प्राप्त शिकायतों के त्वरित निराकरण हेतु hpp://mgnrega.cg.gov.in/ में ऑनलाईन व्यवस्था की गई है।
5. पारदर्शिता तथा realtime mis अद्यतन करने के उद्देश्य से मनरेगा योजना के अंतर्गत e-master roll का प्रयोग किया जा रहा है।
6. मनरेगा अधिनियम की धारा 27 के अंतर्गत योजना के क्रियान्वयन से संबंधित शिकायतों के समाधान हेतु जिला स्तर पर 17 लोकपाल की नियुक्ति का प्रावधान किया गया है। वर्तमान में 15 लोकपाल कार्यरत् है।
7. जिलों के लोकपाल द्वारा पारित अवार्ड में .....सुनवाई हेतु राज्य स्तर पर त्रिसदस्यीय अपीलीय प्राधिकरण का गठन प्रक्रियाधीन है।
8. मजदूरी भुगतान में होने वाले विलंब को कम करने के उद्देश्य से e-FMS प्रणाली प्रारंभ की गई है।

संदर्भ :–

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2. cgpanchayat.gov.in
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6. Cgrd.gov.in