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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.

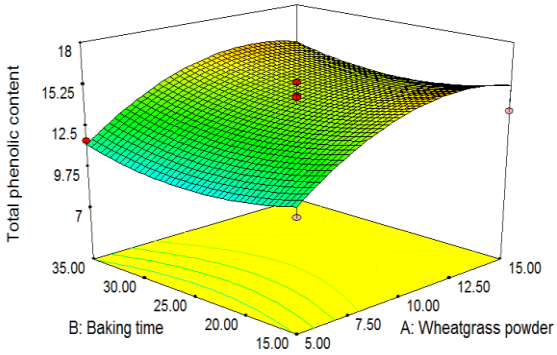

Figure 1. Response surface plot of the effects of wheatgrass powder (g) and baking time (min.) on total phenolic content (TPC) of wheatgrass cupcake.

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.

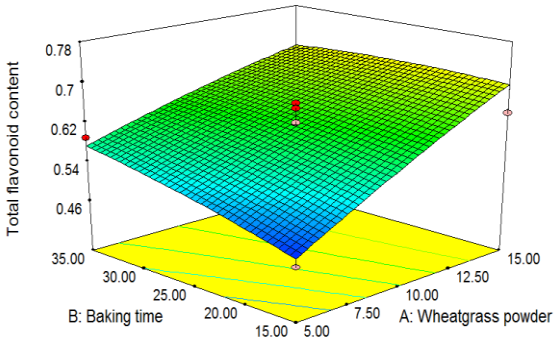

Figure 2. Response surface plot of the effects of wheatgrass powder (g) and baking time (min.) on total flavonoid content (TFC) of wheatgrass

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).

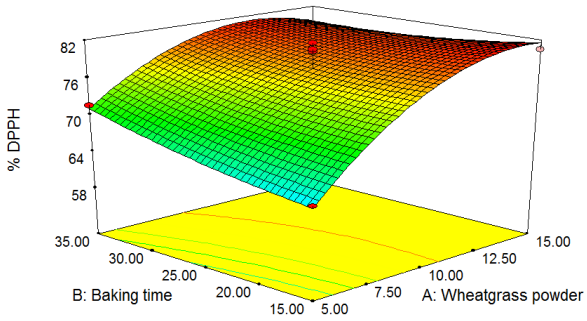

Figure 3. Response surface plot of the effects of wheatgrass powder (g) and baking time (min.) on total % DPPH (TPC) of wheatgrass cupcake.

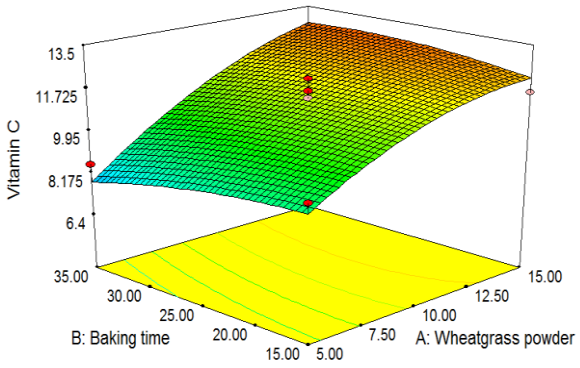

Figure 4. Response surface plot of the effects of wheatgrass powder (g) and baking time (min.) on ascorbic acid of wheatgrass cupcake.

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.

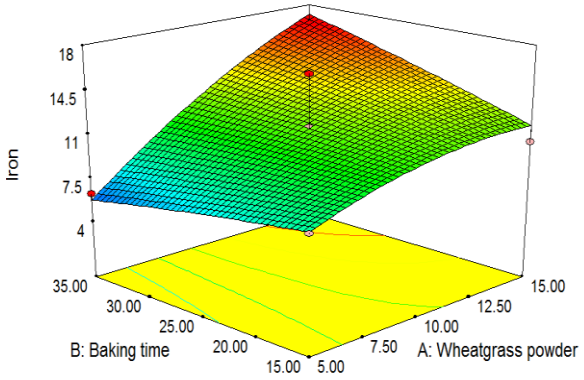

Figure 5. Response surface plot of the effects of wheatgrass powder (g) and baking time (min.) on iron content of wheatgrass cupcake.

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.

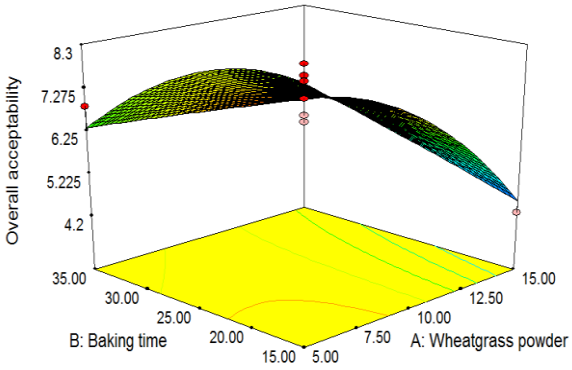

Figure 6. Response surface plot of the effects of wheatgrass powder (g) and baking time (min.) on overall acceptability of wheatgrass cupcake.

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.

5.References

AOAC, Association of Official Agricultural Chemists (2005). *Official Methods of Analysis*, 18th edn. Washington, DC. Association of Official Agricultural Chemists.

Bahorun, T.; Gressier, B.; Trotin, F.; Brunete, C.; Dine, T.; Vasseur, T.; Gazin, TC.; Pinkas, M.; Luycky, M. and Gazin, M. (1996). Oxygen species scavenging activity of phenolic extact from Hawthorn fresh plant organs and pharmaceutical preparation. *Arznein Forsch/Drug Res*., 2, 1-6.

Blois, M.S. (1958). Antioxidants determination by the use of a stable free radical, *Nature* ,4617, 1199–1200.

Borek, C. (2001). Antioxidant health effects of aged garlic extract. *Journal of Nutrition*, 131, 1010-1015.

Bunkar, D.; Jha, A. and Mahajan, A.(2012). Optimization of the formulation and technology of pearl millet based 'ready-to-reconstitute' kheer mix powder, *Journal of Food Science and Technology*, 1, 1-10.

Chan, S.; Lee, C.; Yap, C.; Wan, W. and Ho, C. (2009). Optimization of extraction conditions for phenolic compounds from limaupurut (Citrus hystrix) peels. *International Food Research Journal*, 16, 203-213.

Chauhan, M. (2014). A pilot study on wheat grass juice for its phytochemical, nutritional and therapeutic potential on chronic diseases. *International Journal of Chemistry*, 2(4), 27-34.

Dey, S.; Sarkar, R.; Ghosh, P.; Khatun, R.; Ghorai, K.; Choudhari, R.; Ahmad, R.; Gupta, P.; Mukopadhya, S. and Mukopadhya, A. (2006). Effect of Wheat grass Juice in supportive care of terminally ill cancer patients- A tertiary cancer centre Experience from India. *Journal of Clinical Oncology ASCO Meeting Proceedings Part I*; 18(1), 8634.

Deora, N. S., Deswal, A., Dwivedi, M., & Mishra, H. N. (2014). Prevalence of coeliac disease in India: A mini review. *Internation Journal Latest Research Science Technology*, *3*(10), 58-60.

Eichner, K. (1981). Antioxidant effect of Maillard reaction intermediates. *Progress in Food and Nutrition Science*, 5 (1), 441-451.

Jerome, R. E., Singh, S. K., & Dwivedi, M. (2019). Process analytical technology for bakery industry: A review. *Journal of Food Process Engineering*, *42*(5), e13143.

Kakade, M.L.; Rackis, J.; McGhee, E. and Puski, G. (1974). Determination of trypsin inhibitor activity of soy products: a collaborative analysis and improved procedure. *Cereal Chemistry*, 51, 376-382.

Manzocco, L., Calligaris, S., Mastrocola, D., Nicoli, M. C., & Lerici, C. R. (2000). Review of non-enzymatic browning and antioxidant capacity in processed foods. *Trends in Food Science & Technology*, 11, 340-346.

Marwaha, RK.; Bansal, D.; Kaur, S. and Trehen, A. (2008). Wheatgrass juice reduces the transfusion requirement in patients with thalassemia major: A pilot study. *Indian Pediatrics*., 41, 716-720.

Nencini, C., Menchiari, A., Franchi, G. G., & Micheli, L. (2011). In vitro antioxidant activity of aged extracts of some Italian Allium species. *Plant Foods for Human Nutrition*, 66 (11), 16.

Oyaizu, M.(1986). Studies on products of browning reactions: antioxidative activities of products of browning reaction prepared from glucosamine. *Japanese Journal of Nutrition*, 44: 307–315.

Pulido, R.. Bravo, L., Sauro, F. C. (2000). Antioxidant activity of dietarypolyphenols as determined by a modified ferric reducing/antioxidant power assay, *Journal of Agriculture and Food Chemistry*. 48, 3396–3402.

Ranganna, S. (2005). Sensory evaluation. In: Hand Book of Analysis and Quality Control for Fruit and Vegetable Products, Tata McGraw Hill Education Private Ltd, New York, USA.

Rifna, E. J., Singh, S. K., Chakraborty, S., & Dwivedi, M. (2019). Effect of thermal and non-thermal techniques for microbial safety in food powder: Recent advances. *Food Research International*, 108654.

Sadasivam, S. and Manickam. (2005). A Phenolics, Anti- Nutritional Factors. Biochemical Methods. Tamil Nadu Agricultural University: New Age International Private Limited, New Delhi, India, 205-216.

Stratil, P., Klejdus, B., & Kubán, V. (2006). Determination of total content of phenolic compounds and their antioxidant activity in vegetables e evaluation of spectrophotometric methods. *Journal of Agricultural and Food Chemistry*, 54 (60), 616.

Swati, P.; Sushma, D.; Indira, R.; Alka, G. and Mamta, D. (2010). Multitude potential of wheatgrass juice (Green Blood): An overview. *Chronicles of young scientists*.1(2), 23- 28.

Tripathi, R., Sharma, D., Dwivedi, M., Rizvi, S. I., & Mishra, N. (2017). Wheatgrass incorporation as a viable strategy to enhance nutritional quality of an edible formulation. *Annals of Phytomedicine*, *6*(1), 68-75.

Udeme, J. J. I., et al., (2014). Microbiological, Nutritional, and Sensory Quality of Bread Produced from Wheat and Potato Flour Blends . *Hindawi Publishing Corporation International Journal of Food Science*.

Varnalis, A.I., Brennan J.G., MacDougall D.B., Gilmour S.G., (2004). Optimisation of high temperature puffing of potato cubes using response surface methodology. *Journal of Food Engineering* 61, 153-163.

Walters, R . (1992). The Alternative Cancer Therapy Book. *New York Avery Publishing Group*, 299-308.

Watts, B.M., Ylimaki, G.L., Jeffery, L.E. and Elias, L.G. (1989) Basic Sensory Methods for Food Evaluation. *International Development Research Center*, Ottawa, 60-63.