

Development and optimization of wheatgrass-based instant soup mix using response surface methodology

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Abstract

Introduction: Wheat grass is the shoot of *Triticum aestivum* Linn. belongs to the family Gramineae, and possesses high chlorophyll content, essential vitamins, minerals, vital enzymes, aminoacids, dietary fibers etc., It has been shown to possess anti-cancer, anti-ulcer, antioxidant and anti-arthritis activity due to the presence of biologically active compounds, and minerals. Therefore, the present study has been proposed to develop optimized wheatgrass-based instant soup mix using response surface technology as a statistical tool.

Materials and Methods: Response Surface Methodology (RSM) is a statistical tool which can be used to determine the best combination of ingredients to optimize overall acceptability of any food product. Ingredients such as lyophilized wheatgrass powder, tomato powder, and wheat bran have been selected as independent variables, while overall acceptability (OAA) and consistency were considered as dependent variables. Physicochemical, rheological, antioxidant activity, sensorial parameters were determined for the optimized instant soup mix and compared with the commercial sample.

Results: The results indicated that lyophilized wheatgrass significantly increased ($P < 0.05\%$) the nutritional contents, namely protein, fiber, total polyphenols, and flavonoids. Further, studies on high-performance liquid chromatography reveal that the soup mix consists of a high amount of sinapic acid. Moreover, the study revealed that the developed soup mix is rich in antioxidant activity with good overall acceptability.

Conclusion: The developed combination of wheat-based soup mix efficiently can be consumed as a health booster.

Keywords: DPPH, response surface methodology, sensory evaluation, sinapic acid, wheatgrass-based instant soup mix

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INTRODUCTION

Instant foods are convenient food products that are easy, hygienic, free from microbial contamination, and quick to prepare.^[1] Soups are generally consumed as appetizers and they play an important role in balancing the nutrients, particularly in patients whose intake of solids is poor due to several pathological reasons. Instant soups are a group of dried foods, which could protect from enzymatic and oxidative

spoilage, further provide flavor stability at room temperature for a long time with low unit volume. Moreover, instant soup powders are well adapted to institutional use for military rations as well. Instant soup mixes are available in the form of dry granules, dry compacted cubes, or as a concentrated liquid, which could be in the form of ready to reconstitute Ready to Reconstitute (RER) into hot soups with water dilution at the desired concentration. It contains high nutritive values such as the presence of vitamins, minerals,

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and also rich in fiber. Furthermore, they are ready to reconstitute in a short time. Wheat (*Triticum species*), annual cereal grass of the Gramineae (Poaceae) family, is the world's largest edible cereal grass crop and commonly referred to as wheatgrass. Since ancient times, wheatgrass has been used to treat various diseases and continues to be used as a health tonic. Tender wheatgrass is a potent source of various nutrients such as proteins, minerals, vitamins, active enzymes, and phytochemicals such as alkaloids, glycosides, saponins, steroids, tannins, and flavonoids.^[2] Medical application of wheatgrass ranges from the minor sickness to life-threatening diseases such as cancer. Antioxidant effects of wheatgrass compensate for the prevention of certain degenerative diseases such as diabetes and cardiovascular disease.^[3] It has been found to be effective in different circumstances, such as anemia, asthma, obesity, eczema, constipation, inflammation of the kidneys, and common cold. Wheatgrass stimulates metabolism, restores alkalinity in the blood, and its abundance of alkaline minerals helps reduce overacidity in the blood. Wheatgrass is also a detoxifier and aims to preserve balanced cells.^[4] Wheatgrass juice (WGJ) also contains several flavonoid products, such as sinapic acid, luteolin, apigenin, and quercetin. Due to the content of these compounds, WGJ helps to cure inflammatory bowel diseases in a way.^[5] Antioxidant-rich ingredients like tomatoes in the soup will further add variety, provide health benefits, and make food appetizer. Even though the ripe tomato contains about 94% moisture, it is an excellent source of minerals and vitamins. Tomato contains large amounts of Vitamin C and A, providing 40% and 15% of the daily value, respectively. Moreover, the lycopene, a red pigment found in tomatoes, acts as an antioxidant and neutralizes the free radicals that can damage cells. Several other ingredients such as salt, black pepper, onion powder, and wheat bran have been used in this study to prepare an acceptable soup mix. The present study was undertaken to develop a wheatgrass-based instant soup mix by optimizing ingredients by response surface methodology (RSM) with desired sensorial, nutritional, and functional properties with antioxidant activity.

MATERIALS AND METHODS

Raw material selection

The present investigation was carried out in the Defence Food Research Laboratory (DFRL), Mysuru. Wheat seeds of variety HD 3086 were obtained from the Indian Agriculture Research Institute, Delhi, and sowed in DFRL garden area and young wheatgrass was collected on the 9th day when it was 10 cm in height from the ground. The WGJ was extracted and it was lyophilized to make it powder and packed immediately in polypropylene pouches. Fresh mature tomatoes and onions were purchased from the local vegetable market, Mysuru. All other ingredients such as black pepper and salt were also purchased from local stores. All the other chemicals were procured from Sigma Aldrich, India.

Experimental design

The central composite rotatable design (CCRD) of RSM was used for designing the experimental combinations for the development of wheatgrass-based instant soup mix and sensory attributes using software, State-Ease (Design-Expert version 10.0.10). Lyophilized wheatgrass powder, tomato powder, and wheat bran were selected as independent variables and overall acceptability (OAA) and consistency were considered as responses. Design of experiments for the development of wheatgrass-based instant soup mix is shown in Table 1. The factorial design with 20 sets of experiments

Table 1: Central composite design for wheatgrass-based instant soup mix

Wheatgrass powder (g) χ^1	Tomato powder (g) χ^2	Wheat bran (g) χ^3
0.80	2.81	0.40
1.25	4	0.62
1.25	4	0.62
2	4	0.62
1.25	4	0.62
0.5	4	0.62
1.25	2	0.62
1.25	6	0.62
1.69	5.18	0.84
1.69	2.81	0.40
1.25	4	0.62
0.80	2.81	0.84
0.80	5.18	0.84
0.80	5.18	0.40
1.69	2.81	0.84
1.69	5.18	0.40
1.25	4	0.25
1.25	4	0.62
1.25	4	0.62
1.25	4	1

consisted of seven factorial points, six central points, and seven axial points.^[6] All variables of the polynomial regression at a significance level of $P < 0.05$ were included in the model and the coefficient of determination (R^2) was generated to assess the accuracy of the model.^[7] The response surfaces were obtained from the equation of the second-order polynomial, using the values of each independent variable to the maximum quadratic response.^[8]

First-order linear equation (1)

$$Y = \beta_0 \pm \sum_{i=1}^n \beta_i \times i$$

Second-order polynomial equation (2)

$$Y = \beta_0 + \sum_{i=1}^n \beta_i x_i + \sum_{i=1}^n \beta_{ii} x_i^2 + \sum_{i=1}^n \sum_{j=1}^n \beta_{ij} x_i x_j$$

where 0 was the value of the fitted response at the center point of the design, i.e. point (0,0,0) in case of wheatgrass powder, tomato powder, and wheat bran; I, II and III were quadratic regression terms, respectively, and n denotes the number of independent variables.

Development of wheatgrass-based instant soup mix

Ripe fresh tomatoes were sorted, cleaned, and sliced into with 5 mm thickness then pretreated by dipping with 0.2 g/100 g KMS and 1% $CaCl_2$ (1:1; w/w)^[9] in an equal quantity of water for 10 min.^[10] Tomato slices were dried in a cabinet drier at 60°C for 18 h then cooling, grinding, and packing in polypropylene pouches (PPE). The dehydrated onion was prepared by drying in a cabinet drier for 10 h at 60°C after pretreatment with 1% KMS potassium metabisulfate solution then after drying, grinding, sieving, and storing in polypropylene pouches.^[11] The soup ingredients such as lyophilized wheatgrass powder, tomato powder, and wheat bran were considered as variables for their optimization levels using RSM and the fixed ingredients combination, i.e. fenugreek powder, onion powder, garlic powder, black pepper powder, salt, and cumin powder were decided for the instant soup mix.

Measurement of physicochemical analysis

Proximate composition

Moisture, crude fat, and ash content was analyzed by AOAC methods.^[12] Crude protein ($N \times 6.25$) was determined by adopting the micro-Kjeldahl method. The amount of carbohydrate was assessed using the difference method. Water activity (aW) was analyzed in LabMaster water activity analyzer (Novasina AG, Lachen, Switzerland) with temperature control of 25°C. The degree of caking was measured using the method described by Pisecky.^[13] Solubility was determined as per the method by Milton.^[14]

Measurement of color

The color was measured using ColorFlex spectrophotometer (HunterLab Technologies Inc., VA, USA). The instrument was precalibrated with black and white reference tiles before each analysis. Sample cell containing the homogenized sample was placed above the light sources after sensor calibration and postprocessing L^* , a^* , and b^* values along with other parameters were recorded and analyzed using easy match QC (HunterLab Technologies Inc., VA, USA). About three readings were taken and average values were calculated. The CIELAB (L^* , a^* , b^*) color scale was used for the analysis of the samples and products.^[15]

Measurement of viscosity

Rheological measurements such as viscosity and shear rate of optimized soup mix were measured using MCR 100 controlled stress rheometer (Paar Physica, Anton paar, GmbH, Germany) equipped with coaxial cylinders (CC 27) and the radii ratio of coaxial cylinders was 1.08477. The sample was placed in a small adaptor and at constant temperature (25°C). Viscosity and shear rate were obtained directly from the instrument spindle which was selected for the measurement. All the measurements were carried out in triplicate and the fresh sample was used in each measurement.

Determination of antioxidant activity

Antioxidant activity was the determination by DPPH free radical scavenging activity, lyophilized wheatgrass powders were dissolved in (10 mg) 100 ml of methanol for 2 h in the dark. Freshly prepared 5 ml of DPPH (0.1 mM) was added to 100 μ l extract and incubated for 30 min. The absorbance of DPPH was taken at 517 nm. Methanol was taken as solvent blank. The reduction of the purple color of the DPPH solution to pale color was considered as a percentage of inhibition.^[16] Inhibition concentration was calculated for the interpretation of the results from the DPPH method. It was the amount of sample necessary to decrease the absorbance of DPPH by 50%. Ascorbic acid was used as standard.

Total phenolics and flavonoid content

The total phenol content was measured with the Folin-Chloride-Dipyridyl, spectrophotometrically.^[17] Aluminum chloride method was used for flavonoid content estimation.^[18]

Determination of individual polyphenols by high-performance liquid chromatography

Identification of individual polyphenols was done by a method of Hartl and Stenzel 2007^[19] with slight modifications using high-performance liquid chromatography (HPLC) analysis with Agilent chromatography system. The best solvent system as a mobile phase for the study consists of methanol: water with 0.1% trifluoroacetic acid (80:20, w/w), with a flow rate of 1.0 ml/min and detection range from 200 to 400 nm at ambient temperature. The HPLC peaks of analytes were confirmed and quantified with known reference standards by comparing their retention times.^[20]

Statistical analysis

The results are presented as the mean \pm standard deviation of triplicate observations. All the data were analyzed for analysis of variance (ANOVA) using randomized design with the least significant difference at 0.05 levels by using the Statistical package for the socialsciences (SPSS) 13 Windows Students version software.

RESULTS AND DISCUSSION

Response results of wheatgrass-based instant soup mix

The CCRD of RSM was used for designing the experimental combinations for the development of wheatgrass-based instant soup mix; the designing parameters are tabulated in Table 1. Ingredient combinations such as wheatgrass powder, tomato powder, and wheat bran were taken as independent variables. Sensory attributes of the food product were the main standard to consider for the determination of OAA of the product. Experimental results of independent variables are shown in Table 2. Central composite design results were used to fit the second-order polynomial equation. Regression analysis of two responses such as OAA and consistency was conducted by fitting the quadratic model. ANOVA was calculated and model statistics for all the responses are shown in Table 2. All responses showed highly significance and fitted with a quadratic model ($P < 0.05$). The effect of variations in the levels of independent variables on three responses has been depicted as three-dimensional response plots as desirability, OAA, and consistency in Figure 1a-c. The results were revealed that the levels of lyophilized wheatgrass powder are having the highest impact and then followed by tomato powder and wheat bran levels on desirability and OAA.

Table 2: Responses of dependent variables for wheatgrass-based instant soup mix

Serial number	OAA (year 1)	Consistency (years 2)
1	7	0.0356
2	7.2	0.0451
3	7.2	0.0481
4	6*	0.0281*
5	7	0.0486
6	7.2	0.0567
7	7.3	0.0362
8	7.8	0.0545
9	6.5	0.0421
10	6.5	0.0315
11	6.8	0.0325
12	7.6	0.0721
13	8.3**	0.0767**
14	7.7	0.0545
15	6.8	0.0362
16	6.5	0.0175
17	6.5	0.0467
18	6.9	0.0452
19	7.2	0.0486
20	7.2	0.0723
Mean \pm SD	7.06 \pm 0.18	0.0464 \pm 0.015
CV	2.53%	18.25
R2	0.9146	0.7405
Adjusted R ²	0.8896	0.6919
Predicted R ²	0.7296	0.5566
Adequate precision	17.956	13.015
Press	1.48	1.96

*Lower values; **Higher values. SD: Standard deviation, CV: Coefficient of variation, OAA: Overall acceptability

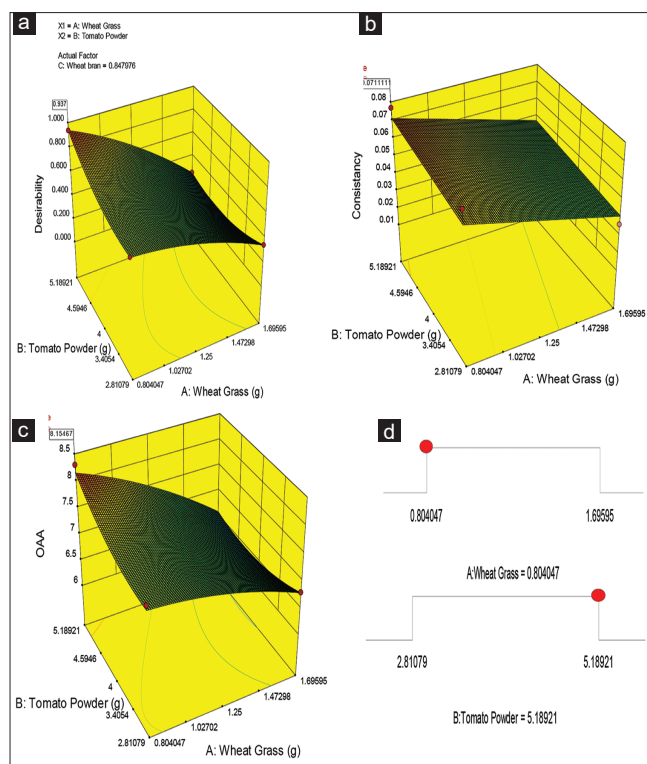


Figure 1: Effect of selected variables on the development of instant soup mix (a) desirability, (b) consistency, (c) overall acceptability, and (d) selected range of variables

Table 3: Comparison of predicted and actual values of selected responses

Responses	Predicted value	Actual value
Sensory score (OAA)	8.15±0.01 ^a	8.18±0.01 ^a
Consistency (Pa/s)	0.071±0.02 ^a	0.068±0.03 ^a

Significance difference was determined at a $P < 0.05$. OAA: Overall acceptability, Pa/s: Pascal /Second

Table 4: Proximate composition, ColoroFlex, physicochemical, and nutritional properties of optimized wheat-based instant soup mix in comparison with commercial instant soup mix

	Commercial soup mix	Optimized soup mix
Proximate composition		
Fat (%)	1.6±0.27 ^b	2.3±0.2 ^a
Protein (%)	3.2±0.01 ^b	5.8±0.02 ^a
Crude fiber (%)	1.2±0.04 ^b	2.2±0.08 ^a
Carbohydrate (%)	87.2±0.5 ^a	82.8±0.3 ^b
Energy (kcal)	361.6±1.2 ^a	354.6±1.8 ^b
Physicochemical properties		
Water activity (a _w)	0.4±0.02 ^a	0.3±0.02 ^a
Caking index (%)	5.1±0.3 ^b	6.2±0.3 ^a
Solubility (%)	87.4±0.2 ^a	80.4±0.3 ^b
pH	4.2±0.02 ^b	4.8±0.03 ^a
Hunter ColoriFlex values		
L*	66.3±0.82 ^a	47.1±0.08 ^b
a*	10.8±0.24 ^a	9.4±0.9 ^b
b*	14.6±0.39 ^b	22.5±0.49 ^a

L* Indicates lightness, a* is the red/green coordinate and b* is the yellow/blue coordinate Significance difference was determined at ^a $P < 0.001$ and ^b $P < 0.05$

Optimization of ingredients and assessment of scores
Responses were optimized using Design-Expert version 10.0.10.

Optimization of the independent variable levels, i.e., lyophilized wheatgrass powder, tomato powder, and wheat bran, was achieved based on the maximization of the responses (OAA and consistency) and suitable desirability was considered for optimized ingredient levels. Optimized levels of wheatgrass-based instant soup mix variables were lyophilized wheatgrass powder (0.8 g), tomato powder (5.18 g), and wheat bran (0.84 g), and responses were found to be OAA (8.15) and consistency (0.071 pa/s). Product was developed with optimized levels of variables and responses were analyzed and verified with the predicted values [Table 3]. It showed that actual values were in line with the predictions. Therefore, the above levels of ingredients were recommended for the optimization of the product. Formulae for the predicted values for the attributes as follow:

- $OAA = +7.05 - 0.46A + 0.14 \times B + 0.20 \times C - 0.21 \times C - 0.21 \times AB - 0.11 \times AC - 0.037 \times BC - 0.13 \times A^2 + 0.20 \times B^2 - 0.046 \times C^2$
- $CONSISTENCY = +0.046 - 0.012 \times A + 3.381E - 003 \times B + 9.596E - 003 \times C.$

The proximate composition of the wheatgrass-based instant soup mix is studied and compared with commercial soup mix available in the market. Comparative results of wheatgrass-based instant soup mix and commercial soup mix are given in Table 4. The results showed that wheatgrass-based soup mix showed a statistically significant difference at $P < 0.05$ with commercial soup mix in the studied parameters such as protein and fiber. Further, the percentage of carbohydrate and energy was significantly less content observed in wheatgrass-based instant soup mix when compared to a commercial sample ($P < 0.05$). Our study was in line with previous observations.^[21]

Results of physicochemical characteristics of optimized soup mix were compared with commercial soup mix and tabulated in Table 4. Water activity is a major parameter in relation to the chemical stability of dried food products and important parameter in determining the shelf life. From the study, it is clear that there was no statistically significant difference in water activity (a_w) ($P > 0.05$) in both the samples. Caking takes place because of the transformation of powders into undesirable lumps ranging from small and soft aggregates that can be broken easily to hard lumps resulting in loss of flowability. There was a statistically significant difference in the caking index, solubility, and pH ($P < 0.05$) in the optimized sample when compared with the commercial sample. The reason may be due to the absence of anticaking agents in the optimized sample. These results coincide with the previous studies.^[22]

Color is one of the most important quality attributes of dried food products. Results of the study indicated that the addition of lyophilized wheatgrass powder significantly decreased the lightness (L*) and redness (a*) and significantly increased yellowness (b*) in optimized soup mix in comparison with the commercial sample [Table 4]. The reason may be due to the green color of wheatgrass powder and increase in yellowness may be due to the addition of fenugreek powder to optimized soup mix than commercial soup mix. Our study is in line with a previous study.^[23]

Rheological behavior of soups

Viscosity is an important characteristic of liquid foods. In soup making, viscosity is the index of thickness. The relationship between

Table 5: Sensory attributes of optimized wheat-based instant soup mix in comparison with commercial instant soup mix

Sensorial attributes	Commercial soup mix	Optimized wheatgrass based instant soup mix
Colour	8.3±0.1 ^a	8.12±0.24 ^b
Flavour	8.2±0.2 ^a	8.2±0.33 ^a
Taste	8.1±0.22 ^a	8.0±0.24 ^a
Consistency	8.3±0.15 ^a	8.0±0.18 ^b
OAA	8.2±0.05 ^a	8.2±0.08 ^a

Significance difference was determined at ^a*P*<0.001 and ^b*P*<0.05. OAA: Overall acceptability

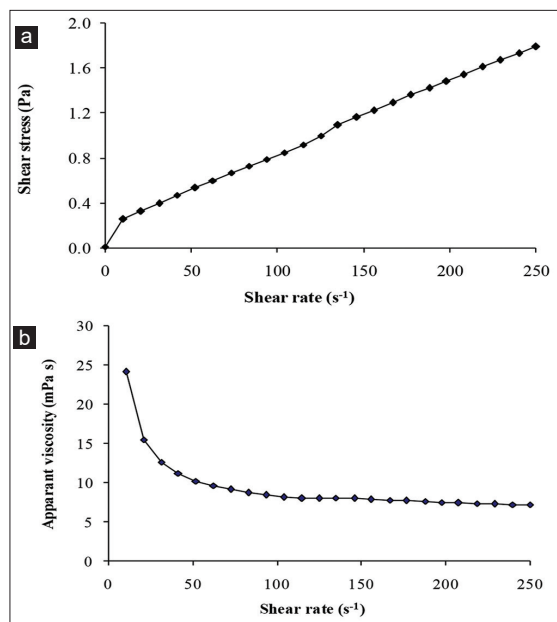


Figure 2: Rheological behavior of wheat based instant soup mix. (a) Shear stress (pa) and (b) viscosity (mpa/s)

viscosity and shear rate is used to classify foods into Newtonian, non-Newtonian, pseudoplastic, dilatant, rheopectic, and thixotropic. Such classification is known to be useful in processing, quality control, sensory evaluation, and structural analysis. Figure 2a shows the variation of shear stress (Pa) concerning the change in shear rate (s⁻¹) and Figure 2b shows the variation of viscosity (mPa s) concerning the change in shear rate (s⁻¹) of soup. The figures indicate that the shear stress increases with a shear rate without the existence of yield stress, whereas viscosity decreases with an increase in shear rate which indicated that the flow is non-Newtonian pseudoplastic behavior. The power-law model is suitable to describe the variation of shear stress to shear rate and equation represented as:

$$\tau = k(\dot{\gamma})^n$$

where τ is shear stress (pa), shear rate (s⁻¹), k is the consistency index Pa sⁿ Figure 1d, and n is the flow behavior index (-). The parameters of the model such as consistency coefficient, flow behavior index, and apparent viscosity at a shear rate of 125 s⁻¹ for comparison purpose and coefficient of determination (R²) values were reported. The consistency coefficient (k) varies from 0.008 Pa

sⁿ to 0.543 Pa sⁿ, whereas the flow behavior index (n) varies from 0.490 to 0.950 which indicated the flow behavior is non-Newtonian pseudoplastic behavior and the apparent viscosity (η_{125}) at a shear rate of 125 s⁻¹ varies from 6.3 mPa s to 53.2 mPa s. The results coincide with the previous studies.^[24]

Individual polyphenols by high-performance liquid chromatography

High-performance liquid chromatogram analysis of individual polyphenols is shown in Figure 3. Most of the phenolic compounds were present in high concentration in the present optimized soup mix. It shows that the highest amount of sinapic acid, i.e., 7.68 mg/g followed by gallic acid, tannic acid, ferulic acid, coumaric acid, etc. Further, other polyphenols such as epigallocatechin gallate, etc., were quantified in lower concentrations. All these polyphenols have been reported with various pharmacological activities in animal experimental models.^[25] Sinapic acid is an anti-inflammatory agent; therefore, WGJ may help to cure inflammatory bowel diseases. Previous studies^[26] are supporting our present results.

Antioxidant analysis

Antioxidant analysis through DPPH radical scavenging results of wheatgrass-based optimized instant soup mix was compared with commercial soup mix, our optimized wheatgrass-based instant soup mix shown significantly more (*p*< 0.05%) than commercial soup mix. Antioxidant activities, such as total polyphenols, total flavonoids, and DPPH was 5.43mg/g, 1.13 mg/g, IC50 activity 10 mg/g respectively for optimized wheat grass based instant soup mix and for commercial soup mix was 1.22 mg/g, 0.6 mg/g, IC50 12.06 mg/g respectively. This may be due to the presence of higher antioxidant activity in lyophilized wheat grass powder. Phenolic compounds and flavonoids in wheat grass powder were contributed for antioxidant capacity. The presence of antioxidants might be the reason to enrich the product with nutritional and functional value. Recent studies are supporting our present antioxidant activity results and in line with other studies.^[27]

Sensory evaluation is an important tool for food acceptability. It is useful in judging the product improvement and quality maintenance in new product development. Dry soup mix should possess desired quality, dominant flavor, the aroma of the ingredients used in it. The product must be free from the off-flavor, off-taste, bitterness, and faulty texture. The sensory score of optimized instant soup mix and commercial soup mix samples is tabulated in Table 5. From the results, it is clear that there was a statistically significant difference in color and consistency (*P* < 0.05), and no statistically significant difference was observed for flavor, taste, and OAA (*P* > 0.05) when compared to optimized wheatgrass-based soup mix with commercial soup mix. The results coincide with the study conducted.^[28]

CONCLUSION

In the present study, ready-to-reconstitute lyophilized wheatgrass-based instant soup mix has developed. Moreover, the developed instant soup mix has several beneficial properties when compared to commercially available samples. The important properties of developed soup mix include ready to reconstitute form followed by sinapic acid and antioxidant-rich with nutrients. Further, this is one of the most emerging processing techniques

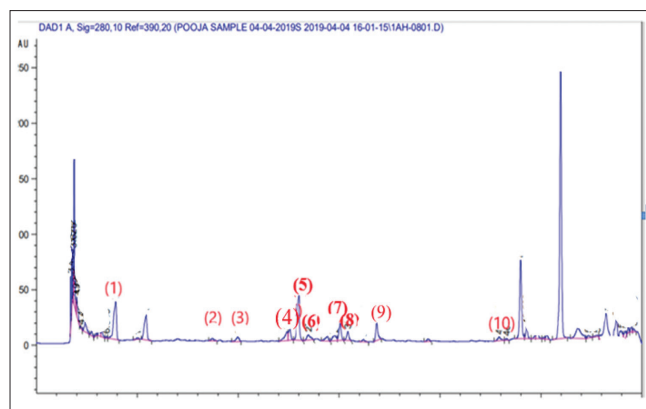


Figure 3: Chromatogram of individual polyphenols by high-performance liquid chromatography of optimized wheatgrass soup mix. (1) gallic acid, (2) chlorogenic acid, (3) epigallocatechin gallate, (4) catechin, (5) sinapic acid, (6) coumaric acid, (7) tannic acid, (8) ferulic acid, (9) rutin hydrate, and (10) Quercetin

that can be applied for new product development. RSM helps in the standardization of the soup mix with various combinations of ingredients. RSM used in the study showed a quadratic response surface as the best fit for prediction with minimum standard error and maximum r^2 . The studies indicated that the sinapic rich instant soup mix can be developed from lyophilized wheatgrass powder and it is value added and superior when compared to commercial instant soup mixes as far as the contents of protein, fiber, total phenolics, total flavonoids, and DPPH activity. Therefore, this studied wheatgrass-based instant soup mix can be recommended as a health booster for consumers with good nutritional and functional value.

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Conflicts of interest

There are no conflicts of interest.

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