Effects of Process Variables On Physical and Antioxidant Properties of Honey Powder With Mungbean-Wheat Flour Based Cookies and Its Optimization

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Abstract - The purpose of this study was to investigate the effect of process variables on the properties of honey powder enriched cookies. The effects of honey powder, mungbean flour and wheat flour on product responses viz. spread ratio (SR), Breaking strength (BS), Antioxidant activity (AOA), Total phenolic content (TPC) and Total flavonoids content(TFC) was studied using response surface methodology. The blend was cookies at different honey powder (15-25%), mungbean flour (10–30%) and wheat flour (70-90%).The regression models for spread ratio, breaking strength, antioxidant activity, total phenolic content and total flavonoid content were highly significant. Spread ratio and breaking strength have been found to decrease with increase of honey powder and wheat flour and increased with increase in mungbean flour. Antioxidant activity was significantly affected by honey powder and mungbean flour. TPC and TFC were found to decrease significantly with increase in wheat flour and increased significantly with increase in honey powder and mungbean flour. The optimum values for honey powder, mungbean flour and wheat flour were 15%, 10% and 90% respectively. The desirability was found to be 0.761 for this model.

Keywords:* *Honey powder, Wheat, Mungbean, Optimization, Cookies, Antioxidant activity.

1. INTRODUCTION

Honey is a natural biological product that assimilates of simple sugars (glucose and fructose: 70–80%), water (10–20%), and other minor constituents such as organic acids, mineral salts, vitamins, proteins, phenolic compounds, and free amino acids (1). It is conventionally liked by consumers for its nutraceutical value, characteristic flavor, sweetness, and texture (2). Honey esteemed both enzymatic and non-enzymatic antioxidants, including glucose oxidase, catalase, ascorbic acid, flavonoids, phenolic acids, carotenoid derivatives, organic acids, Maillard reaction products, amino acids and proteins (3-6). It can be used in cookies formulation to increase overall quality of the product and to extend its shelf life. However, liquid honey is viscous and difficult to disperse in the dough, limiting its use in the food industry (7). By contrast, honey powder (dry honey) made from liquid honey can be dispersed easier, and so it has been more widely used in baking industry for improving cookies quality.

Cookies are convenient food products, becoming very popular among both youth and elderly peoples. Some of the reasons for such wide popularity are low cost among other processed foods, varied taste, easy availability, longer shelf life and serve as a vehicles for important nutrients (8-9). It represents the largest category of snack item among baked food products throughout the world (10). Cookies are not considered as staple food as in bread, but may be feasible fiber carriers because of their long shelf life and thus enable large scale production and widespread distribution (11). In many countries, cookies are prepared with fortified or composite flour to increase its nutritive value (12).

In India, wheat is a second major cereal crop after rice with a production of 94 million tons in 2011-12. Soft wheat flour is used in cookies formulation, because of its content of gliadin (a prolamin) and glutamine (glutelin) which under goes hydration in the presence of water, salt and sugar. This protein form a visco-elastic matrix known as gluten, being responsible for the rising nature of dough or permit substantial increase in the volume of baked product of dough and its gas retention capability (13). Legumes are rich in protein and complex hydrocarbons along with presence of appreciable quantities of bioactive ingredients and minerals and it includes some phytochemicals of interest antioxidants, phytosterols and bioactive carbohydrates (14-15). Biological activities of protein correspond to amino acid composition, sequence, size and configuration of peptides. For example, the presence of certain amino acids, such as Trp, Tyr, His, Trp, Tyr, Met, Leu and gly have been reported to exhibit radical-scavenging activities (18-19).

Response surface methodology (RSM) is effective optimization tool wherein many factor and their interaction affecting the response variable, central composite rotatable design was used here for optimization of process variables for making cookies from combination of wheat, mungbean flour and honey powder. Therefore, this study was undertaken with objectives to study Effects of process variables on physical and antioxidant properties of honey powder with mungbean-wheat flour based cookies and its optimization.

2. MATERIAL AND METHODS

2.1 Materials

Mungbean and wheat grains were procured from sangrur (India). Honey Powder was purchased from Pellagic Foods Ingredients Pvt. Ltd., Delhi. Wheat and mungbean were ground in grinder to pass through 60 BSS sieve

2.2 Experimental design

Statistical software Design-Expert 7 (Stat-Ease®, USA) was used to design the experiment. The central composite rotatable design for the three independent variables was performed. The independent variables considered were honey powder, mungbean flour and wheat flour. Dependent variables were spread ratio, breaking strength, antioxidant activity (AOA), total phenolic content (TPC) and total flavonoid content (TFC). Response surface methodology was used to investigate the effect of process variables on the product responses. The independent variable levels like honey powder (15-25 %), mungbean flour (10-30 %) and wheat flour (70-90 %) considered for study were selected on the basis of the preliminary trials. The design required 20 experimental runs with eight factorial points, six star corner points and six centre points. Experiments were randomized in order to minimize the systematic bias in observed responses due to extraneous factors.

2.3 Determination of product responses:

2.3.1 Spread ratio

Spread ratio was measured by dividing the average value of width (W) by average value of thickness (T) of biscuits (20). Width of biscuits was measured by laying six biscuits edge to edge with the help of a scale rotating the 90^0 and re-measuring the width of six cookies in cm and then taking average value. Thickness (T) or height of biscuits was measured by stacking six biscuits on top of one another and taking average thickness (T) of six biscuits in cm.

$$\text{Spread ratio} = \frac{Average\ value\ of\ width(W)}{Average\ value\ of\ thickness\ (T)}$$

2.3.2 Breaking strength

Breaking strength of cookie was measured using the HDP/BS blade. The individual samples of cookies were placed on the platform such that they were supported at two points and the blade was attached to the crosshead of the instrument. The texture analyzer (TA) setting was kept at: Pre-test speed of 2 mm/s, Test speed of 3 mm/s; Post-test speed of 10 mm/s (21). This test simulates the evaluation of hardness by consumer holding the cookie in hands and breaking the same by bending.

2.3.3 Antioxidant activity (DPPH radical scavenging activity)

Antioxidant activity (AOA) was measured using a modified version of the method described by Brand-Williams, Cuvelier, and Berset (22). Sample (100 mg) was extracted with 1 mL methanol for 2hr and centrifuged at 3000g for 10 min. The supernatant (100 μL) was reacted with 3.9 mL of a 6×10^{-5} mol/L of DPPH solution. Absorbance (A) at 515 nm was read at 0 and 30 min using a methanol blank. Antioxidant activity was calculated as % discoloration.

$$\text{DPPH radical scavenging activity}(\%) = [1 - (\frac{\text{Absorbance of sample at t} = 30}{\text{Absorbance of control at t} = 0})] \times 100$$

2.3.4 Total phenolic content (TPC)

The total phenolic content (TPC) was determined according the Foline-Ciocalteu spectrophotometric method (23). Sample (200 mg) was extracted with 4 mL acidified methanol (HCl/methanol/water, 1:80:10, v/v/v) at room temperature

(25^{0}C) for 2 h. Aliquot of extract (200 μL) was added to 1.5 mL freshly diluted (10 fold) Foline-Ciocalteu reagent. The mixture was allowed to equilibrate for 5 min and then mixed with 1.5 mL of sodium carbonate solution (60 g/L). After incubation at room temperature (25 ^{0}C) for 90 min, the absorbance of the mixture was read at 725 nm (Shimadzu, UV-1800, Japan). Acidified methanol was used as a blank. The results were expressed as mg of ferulic acid equivalents (FAE) per gram of sample.

2.3.5 Total flavonoid content (TFC)

The total flavonoid content (TFC) was determined as previously described by Jia, Tang, and Wu (1998) (24). The extract (250 μL) was diluted with 1.25 mL distilled water. Sodium nitrite (75 μL) was added and the mixture was allowed to stand for 6 min. Further, 150 μL of aluminum chloride was added and the mixture was allowed to stand for 5 min. After that, 0.5 mL of sodium hydroxide (40 g/100 mL) was added and solution was mixed well. The absorbance was measured immediately at 510 nm using a spectrophotometer. Catechin was used as standard and results were reported as microgram catechin equivalent (CE)/g of sample.

2.3.6 Statistical analysis and optimization

Responses obtained as a result of the proposed experimental design were subjected to regression analysis in order to assess the effects of honey powder, mungbean flour and wheat flour. Second order polynomial regression models were established for the dependent variables to fit experimental data for each response using statistical software Design-Expert 7 (Stat-Ease®, USA)

$$Y_k = \beta_{k0} + \sum_{i=1}^{n} \beta_{ki} X_i + \sum_{i=1}^{n} \beta_{kii} X_i^2 + \sum_{i=1}^{n-1} \sum_{j=i+1}^{n} \beta_{kij} X_i X_j + e_i$$

Where, Yk represents the measured response; spread ratio, breaking strength, antioxidant activity, total phenolic content, total flavonoid content of cookies and xi (i=1, 2, 3) are independent variables (honey powder, mungbean and wheat flour) respectively and bo, bi, bii and bijare coefficient for intercept, linear quadratic and interactive effects respectively. Data was analyzed by multiple regression analysis and statistical significance of the terms was examined by analysis of variance (ANOVA) for each response. The adequacy of regression model was checked by correlation coefficients. The lack of fit test was used to judge the adequacy of model fit. To aid visualization of variation in responses with respect to processing variables, series of Contour plots were drawn. Numerical optimization was performed to obtain obtained minimum breaking strength and maximum spread ratio, antioxidant activity, total phenolic content and total flavonoid content.

Validation: Samples were prepared using optimized process variables. Predicted and actual value of product response was compared.

Table: 2 Effect of process variables on dependent variables

Std run	Honey Powder (%)	Mungbean Flour (%)	Wheat Flour (%)	Spread Ratio	Breaking Strength (N)	antioxidant Activity (%)	Total Phenolic Content (mg/g)	Total Flavonoid content (mg/g)
1	15	10	70	5.87	55.67	59.22	5.52	0.654
2	25	10	70	5.65	45.9	58.64	6	0.557
3	15	30	70	6.11	66.29	58.41	4.01	0.786
4	25	30	70	6.46	46.25	58.78	8.48	0.918
5	15	10	90	6.21	50.73	62.65	9.01	1.213
6	25	10	90	5.97	46.34	65.82	5.147	0.934
7	15	30	90	5.62	65.26	55.53	4.45	1.057
8	25	30	90	5.94	50.32	60.38	4.12	1.147
9	11.59	20	80	5.86	60.89	55.43	5.733	1.317

10	28.41	20	80	6.02	41.68	60.48	6.45	1.145
11	20	3.18	80	6.1	45.92	62.21	6.02	0.547
12	20	36.81	80	6.25	58.45	60	4.09	0.758
13	20	20	63.18	5.7	63.56	59.52	5.556	0.718
14	20	20	96.81	5.57	58.32	59.32	5.5	1.157
15	20	20	80	6.28	64.23	61.35	3.33	0.961
16	20	20	80	6.22	64.12	62.15	3.02	0.954
17	20	20	80	6.21	61.34	63.14	3.06	0.846
18	20	20	80	6.24	63.11	61.41	3.12	0.759
19	20	20	80	6.25	61.98	61.47	3.45	0.854
20	20	20	80	6.18	63.45	61.42	3.11	0.915

Table: 3 ANOVA and model statistics for the dependent variables

Terms			**Responses**		
Models	**Spread ratio**	**Breaking Strength (N)**	**Antioxidant Activity (%)**	**Total Phenolic Content (mg/g)**	**Total Flavonoid Content (mg/g)**
F - Value	45.89	81.58	8.75	179.74	19.49
P- Value	< 0.0001	< 0.0001	0.0011	< 0.0001	< 0.0001
Mean	6.04	56.69	60.37	4.96	0.91
Standard Deviation	0.054	1.29	1.13	0.19	0.069
C. V. %	0.89	2.27	1.88	3.76	7.58
R Square	0.976	0.986	0.887	0.993	0.946
Adjusted R Square	0.955	0.974	0.786	0.988	0.897
Predicted R Square	0.851	0.932	0.28	0.968	0.793
Adequate Precision	22.45	28.97	12.356	44.33	15.25
Lack of fit	0.0841	0.3459	0.0742	0.3563	0.7033

3. RESULTS AND DISCUSSION

The data on values of physical and antioxidant properties of cookies is presented in table 2. Results of analysis of variance and regression coefficients are shown in table 3 & 4 respectively. The regression models for spread ratio, breaking strength, antioxidant activity, total phenolic content and total flavonoid content were highly significant ($P < 0.001$), with a high correlation coefficient ($R^2 = 0.97$, 0.98, 0.89, 0.99 and 0.93 respectively). None of the models showed significant lack of fit ($P > 0.05$), indicating that all the second-order polynomial models correlated well with the measured data. The predicted R-square was found in reasonable agreement with the adjusted R-square for all the parameters. Adequate precision (signal to noise ratio) greater than 4 is desirable. All the parameters showed high adequate precision (Table 3). Results and observations with respect to the relationship of independent variable with individual dependent variable are being presented as follows

3.1 Spread ratio

Spread ratio is the most important physical property of the cookies. The spread ratio of cookies varied between 5.57 and 6.42 (Table 2). Honey powder, mungbean flour and wheat flour had significant effects on spread ratio ($P < 0.05$). The positive coefficients of the linear terms of honey powder and mungbean flour level indicated that spread ratio increases with increase of these variables, while negative coefficients for wheat flour (Table 4) indicated that spread ratio decrease with increase in wheat flour. The interactions among honey powder and wheat flour ($P < 0.05$); mungbean flour and wheat flour ($P < 0.05$), were found to have significantly positive and negative correlation respectively with spread ratio values fig. 1.

The spread mechanism in cookies is a function of the total availability of water (25) and the amount of dissolved sugar. The fiber absorbs more water as compared to other constituents of the flour. Lesser the fiber content in cookies more will be the availability of water for dissolving sugar (26) and hence more will be the cookie's spread ratio. The decrease in spread ratio of supplemented cookies might be due to the availability of more hydrophilic sites that compete for limited free water in biscuit dough prepared from high-protein flours (27-29).

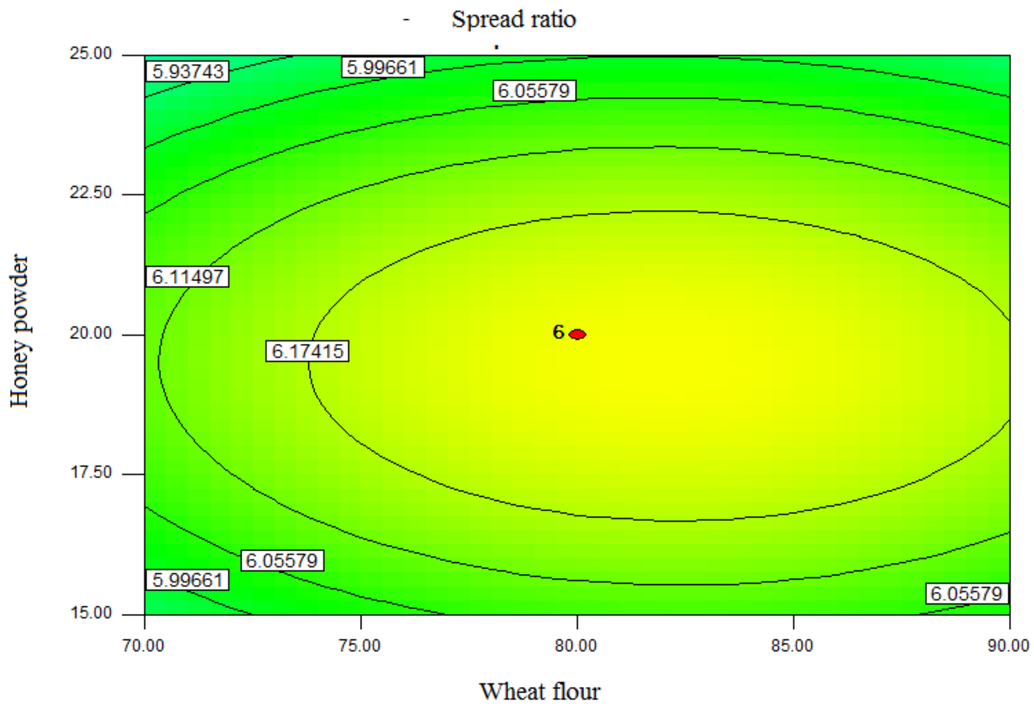

Figure 1: Contour plot showing effect of wheat flour and honey powder on spread ratio

3.2 Breaking Strength

The average initial fracture force is the measure of strength of cookies. The breaking strength is one of the criteria to measure the cookies hardness. The breaking strength of cookies ranged between 41.68 and 66.29 N (Table 2).The breaking strength was influenced significantly by honey powder, mungbean flour and wheat flour content ($p < 0.05$). The positive coefficient of the linear term of mungbean (Table 3) indicated that breaking strength increases with increase in mungbean flour and negative coefficient decreased with increase in honey powder. Other linear term is not significant ($P > 0.05$). All quadratic terms have negative significant effect ($p < 0.05$). Interactions among honey powder and mungbean flour; honey powder and wheat flour had significantly negative and positive correlation ($P < 0.05$).

Results showed breaking strength had a positive correlation with mungbean flour. Increased cookie's breaking strength with increase in mungbean flour might be due to increase in protein content of cookies formulation. McWatter *et al* and Sudha et al (30-31) attributed the harder texture of the cookies to the increased protein content and fiber content and its interaction during dough development and baking. Increased wheat flour with decrease in breaking strength due to wheat flour has a gelatinization temperature range of 56-62^{0}C, but this temperature is markedly altered and increased by physical or chemical modification which affects the strong inter-molecular bonds in the granules structure (32). Gavrilović (33) claims that hardness of honey biscuits depends on starch pasting properties.

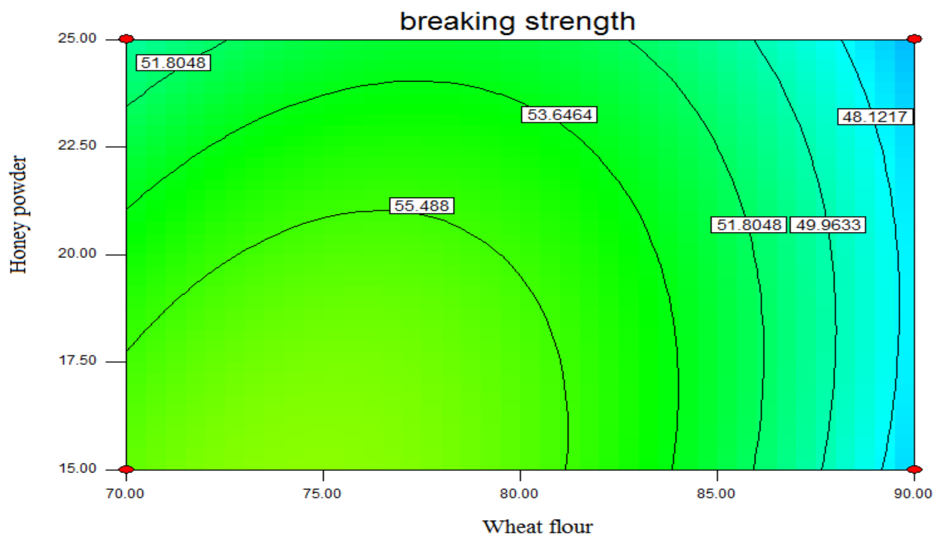

Figure 2: Contour plot showing effect of wheat flour and honey powder on breaking strength

Table: 1 Values of independent variables as per CCRD design.

Independent Variables	**Unit**	**Symbols**	**Levels**				
		Coded	**-1.68**	**-1**	**0**	**+1**	**+1.68**
Wheat flour	(%)	A	63.18	70	80	90	96.82
Mungbean Flour	(%)	B	3.18	10	20	30	36.82
Honey powder	(%)	C	11.59	15	20	25	28.41

Table: 4 Regression coefficients for fitted models

Parameters	**Spread ratio**	**Breaking Strength (N)**	**Antioxidant Activity (%)**	**Total Phenolic Content (mg/g)**	**Total Flavonoid Content (mg/g)**
Intercept	6.227	63.069	61.804	3.177	0.883
Wheat Flour	0.035	-5.9638	1.1937	0.1437	-0.0324*
Mungbean Flour	0.0499	3.7016	-1.2408	-0.5757	0.0662
Honey Powder	-0.0416	-0.7521*	0.6585*	-0.1008*	0.1592
Wheat flour*Mungbean Flour	0.1412	-2.6025	0.3287*	0.9403	0.0747
Wheat flour*Honey Powder	-0.0062*	1.31	1.02875	-1.1428	-0.028*
Mungbean flour* Honey Powder	-0.2087	0.9425*	-1.4862	-0.8196	-0.0545
Wheat Flour2	-0.0854	-4.3577	-1.2459	1.0582	0.116
Mungbean Flour 2	-0.0023*	-4.0395	-0.1322*	0.6918	-0.0884
Honey Powder2	-0.1932	-0.9442	-0.7279	0.8591	0.0123

*Non significant at 5% level of significance ($p<0.05$)

3.3 Antioxidant activity

Antioxidant activity estimation by scavenging of stable radicals, such as the chromogen radical DPPH in inorganic media has been extensively used for comparison of homogeneous series of antioxidants. DPPH assay measures the reducing ability of antioxidants towards DPPH radical. It is one of the short methods that can be used to establish the hydrogen donating potency of compounds (34). The measured DPPH free radical scavenging activity (antioxidant activity, AOA) values of cookies varied between 55.43 and 65.82 % (Table 2). The antioxidant activity increases significantly with decrease in mungbean flour content and increase in honey powder content and wheat flour content. The interaction between mungbean flour content and honey powder ($P < 0.05$) had significant negative correlation with the antioxidant activity. However, wheat flour and honey powder had a positive correlation with the antioxidant activity ($P < 0.05$). Other interaction term is not significant ($P > 0.05$).

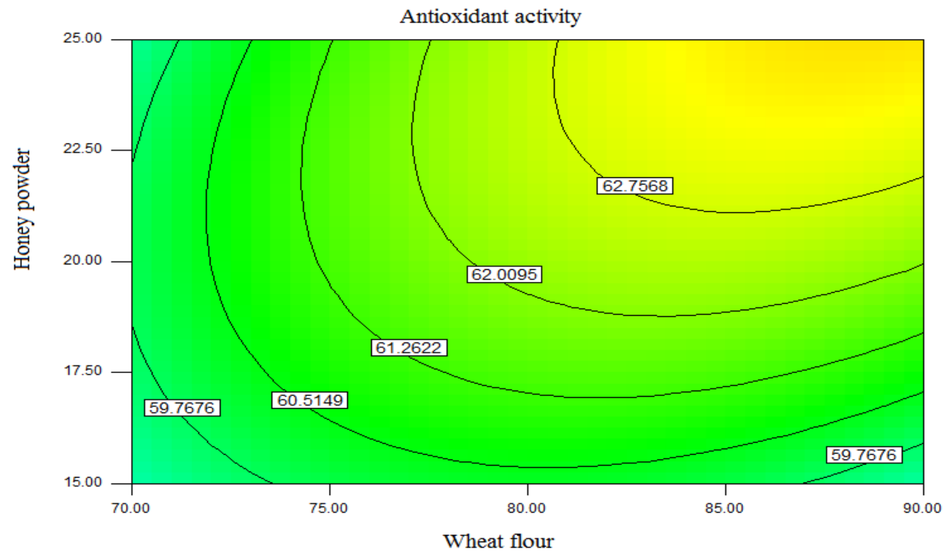

Figure 3: Contour plot showing effect of wheat flour and honey powder on antioxidant activity (% DPPH radical scavenging activity).

Honey powder has been found to be the main factor affecting antioxidant activity of cookies. Increased honey powder leads to increase in antioxidant activity due to be the addition of honey powder which is itself a good source of antioxidants and secondly the formation of Maillard reaction products (MRP). Sharma & Gujral, 2011; Xu & Chang, (35-36) reported that dark colour pigments (brown colour) are produced during the thermal processing of foods due to the Maillard browning. Thermal processing too can alter the antioxidant profile by generating compounds that exhibits increased antioxidant activity. Increase in antioxidant activity due to thermal processing (37-38). These pigments (particularly melanoidins) are extensively known to have antioxidant activity (39). The wheat flour is a good source of antioxidant activity which accounts for 12.3% and blended with mungbean flour increase the antioxidant activity (40). The DPPH radical scavenging activities of the mungbean soup made from cv. Huang, Ming or Mao increased with increasing concentration of mungbean soup (41).

3.4 Total phenolic content

Phenolic compounds are considered as a major group of compounds that contribute to the antioxidant activity of cereal (42). This assay involves the dissociation of phenolic proton which leads to the formation of a phenolate ion which has the capacity to reduce the Folin-Ciocalteau reagent and thus measures the reducing potential of an extract. The measured Total phenolic content (TPC) values of cookies varied between 3.02 mg FAE/g and 9.01 mg FAE/g. Total phenolic content increases significantly with decrease in mungbean flour content and wheat flour content and increase in honey powder content. The interaction between wheat flour and honey powder; mungbean flour and honey powder ($P < 0.01$) had significant negative correlation with total phenolic content of the product. However, wheat flour and mungbean flour had a positive correlation with the total phenolic content ($P < 0.05$).

The total phenolic content decreased with increases in independent variables such as wheat flour content and mungbean content. The phenolic compounds are known to be thermally sensitive and heating beyond 80^0C may destroy or alter their nature (34). Also, the molecular structure of phenolic compounds changes as a result of heating which leads to either reduced chemical reactivity or decreases their extractability due to certain degree of polymerization (43). Angioloni & Collar (44) postulated that during dough making oxidative enzymes such as polyphenol oxidase are activated by hydration of flour and the oxidation may degrade the phenolic compounds. Honey powder content increased with total phenolic content due to honey rich in non enzymatic substances such as ascorbic acids, alfa tocopherol, carotenoids, amino acids etc. (45).

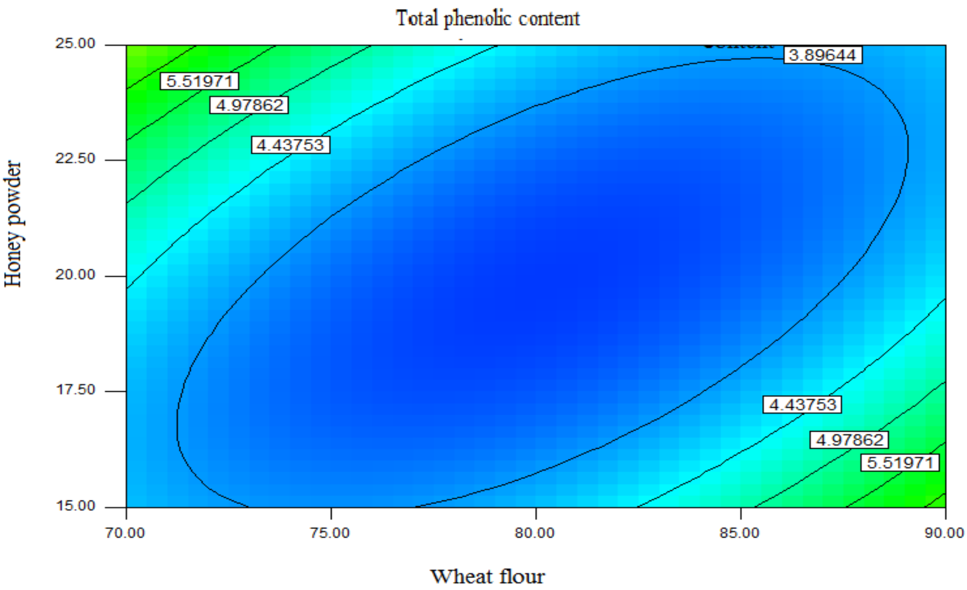

Figure 4: Contour plot showing effect of wheat flour and honey powder on total phenolic content

3.5 Total flavonoid content

Flavonoids are important for human health because of their high pharmacological activities as radical scavengers (46). Flavonoids have shown activity as scavengers of various oxidizing species including superoxide anion, hydroxyl radical and peroxyl radicals (47). Total flavonoids content (TFC) values of cookies ranged between 0.547 mg CE/g and 1.317 mg CE/g. All the independent variables had significant effect on TPC ($P < 0.05$). Increasing wheat flour significantly decreased TFC. However, increase in honey powder and mungbean flour content significantly increased TPC ($P < 0.05$).

The total flavonoid content (TFC) of whole wheat flour was 966 mg catechin equivalents/g where as baking of chapatti decrease total flavonoid content because of the flavonoid is considered to be thermally sensitive and the heat provided to food material during processing destroys the flavonoid, however the extent of degradation of flavonoid depends upon the factors such as the type of substrate and the processing conditions, principally duration of heating (39). Mungbean flour high flavonoids as vitexin and isovitexin which accounts for 1.50 and 1.10 mg of catechin equivalents/g (48). The increase in TFC with the increase in honey powder content may be attributed to the contribution of flavonoids from the honey powder due to honey contain non enzymatic component. These maillards reactions products such as amino acids, carotenoids, and flavonoids are not destroyed at high temperature.

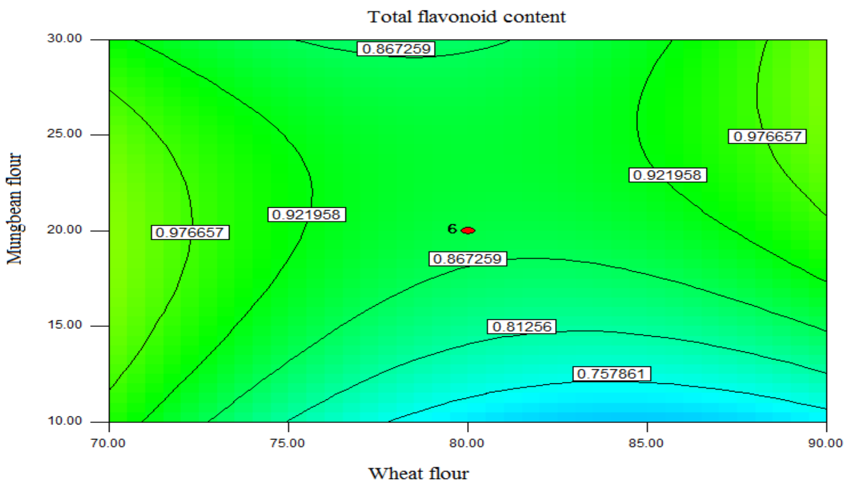

Figure 4: Contour plot showing effect of wheat flour and honey powder on total flavonoid content

OPTIMIZATION AND VALIDATION

Data with respect to predicted and actual values of responses is presented in Table 5. The instant base was then prepared utilized the optimized conditions i.e., 15 % honey powder, 10 % mungbean flour and 90 % wheat flour. Variation in the predicted and actual values was found to be less than 5 per cent.

Table 5: Predicted response vs. actual response

Values			Responses		
	Spread ratio	Breaking Strength (N)	Antioxidant Activity (%)	Total Phenolic Content (mg/g)	Total Flavonoid Content (mg/g)
Predicted Values	6.21	50.38	61.19	9.02	1.21
Actual Values	6.15	52.14	59.85	8.92	1.18
Variations (%)	0.96	3.49	2.18	1.1	2.47

CONCLUSION

RSM revealed the significant effect of all three important process variables (honey powder, mungbean flour and wheat flour) on physical and antioxidant properties of cookies. Within the experimental range, honey powder was the most important factor affecting the physical properties and antioxidant activity of the cookies. Increase in honey powder percentage resulted increase in spread ratio, breaking strength, antioxidant activity, total phenolic content and total flavonoid content. Increase in mungbean flour content resulted increase in spread ratio, breaking strength, antioxidant activity, total phenolic content and total flavonoid content while decreased overall acceptability and total phenolic content. Increase in wheat flour content resulted increase in spread ratio, antioxidant activity, total phenolic content and total flavonoid content while decrease in breaking strength.

The optimum values for honey powder, mungbean flour and wheat flour were observed as 15 %, 10% and 90% respectively. It can be thus concluded that, these process variables suited for cookies prepration.

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