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Nutritional evaluation of wheat and barley composite flour cookies using response surface methodology

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Abstract
Due to consumer awareness of nutrition, the use of barley flour and other components in food products is currently of interest. Since hulless barley flour contains non-glutenous protein, carbohydrates, and soluble fibre, it can enhance the nutritional content of dishes, especially baked goods like cookies. The moisture, fat, protein, carbohydrates, ash, fiber and energy in the cookies varied from 2.4 - 5.25, 22.39 - 25.25, 11.19 - 14.05, 49.39 - 61.59, 0.83 - 1.61, 1.6 - 4.45 percent and 480.22 - 493.84 Kcal respectively. The regression model's results for the above proximate composition were found significant at 5%. The data suggested that the level of barley flour incorporation in the cookies had a positive linear significant effect on the proximate composition.

Keywords: Hulless barley flour, refined wheat flour, guar gum powder, ammonium-bi-carbonate

Introduction
Cookies have become one of the popular snack in the present time due to their low manufacturing cost, convenience, long shelf life, good eating quality also its ability to serve as a carrier for important nutrients (Hooda and Jood, 2005) [6]. Cookies also hold a key position in the baking sector. 82 percent of total bakery production is made up of bread and biscuits, which are regarded as the main bakery products. They are cereal-based foods that often include the three elements fat, flour, and sugar. Aerating agents, flavouring agents, salt, and milk are all common ingredients (Wade, 1988) [12].
Barley (*Hordeum vulgare* L.) is one such cereal which has greater nutrition value. It has both winter and spring, hulled and hulless, and two-row and six-row varieties. It contains starch (65-68%), proteins (10-17%), β-glucan (4-9%), fats (2-3%) and minerals (1.5-2.5%) (Wang *et al.*, 2015) [13]. Hulless barley (*Hordeum vulgare* L. var nudum Hook. f.) forms had a higher content of β-glucan, as well as a higher content of soluble dietary fibres than hulled forms (Fastnaught *et al.*, 1996) [3].

Materials and Methods

Table 1: Following machines and equipments were used in the research work

S. No.	Name of equipments/machine	Purpose	Source of Supply
1.	Hot air oven	For determination of moisture content of the samples	M/S J.K. Sales & Promoters Gole Bazaar, Write Town, Jabalpur (M.P.)
2.	Muffle furnace	For the determination of ash content of flour, and cookies.	M/S J.K. Sales & Promoters Gole Bazaar, Write Town, Jabalpur (M.P.)
3.	Continuous band packaging machine	For storage study the packaging of HDPE, aluminium silver pouches of developed cookies.	M/S Supreti Traders Shop No 6 Methodist Center, OppKartik Hotel Napier Town, Jabalpur
4.	Micro-Kjeldahl Unit	For the determination of protein content of samples viz. flour and cookies.	M/s Pelican Equipments, 2ndFloor, 38 Burket T Nagar, Chennai
5.	Socs Plus, Fat Analyzer	For the determination of fat content of samples viz. flour and cookies.	M/s Pelican Equipments, 2ndFloor, 38 Burket T Nagar, Chennai
6.	Fiber Plus	For the determination of crude fiber content of samples viz. flour and cookies.	M/s Pelican Equipments, 2ndFloor, 38 Burket T Nagar, Chennai

Moisture

The sample of moisture content was calculated using the AOAC (1998) [1] method. The sample (5 g) was collected in a moisture box that had already been pre-weighed, dried at 105 °C for 24 hours in a hot air oven, cooled in desiccators, and weighed. The moisture content of the sample is represented by the moisture box's weight differential.

Calculation:

$$\text{Moisture \%} = \frac{\text{Difference in weight}}{\text{Weight of sample}} \times 100$$

Protein

Using the traditional micro-Kjeldahl digestion and distillation process described in AOAC (1998) [1], the protein content of the sample was measured.

Reagents

1) Catalyst mixture- A mixture of 100 g K_2SO_4, 20 g of $CuSO_4$ and 2.5 g of SiO_2.
2) Sodium hydroxide solution: 40% (w/v).
3) Boric acid solution: 2% (w/v).
4) Concentrated sulphuric acid AR (sp. gr. 1.81)
5) Mixed indicator: 2 parts of 0.2% (w/v) methyl red and 1 part of 0.2% (w/v) methyl blue in absolute alcohol
6) Standard Hydrochloric Acid (0.1N).

Procedure

The sample (2 g) was precisely weighed before being placed to a Kjeldahl flask, with special attention paid to ensuring that the substance did not adhere to the flask's neck. About 2 g of the catalyst combination and 10 ml of concentrated sulfuric acid were added. After that, the flask was heated in the digesting chamber at an angle for around 4-6 hours until the liquid turned transparent (green blue in color).

Distillation

The content in the flask were allowed to cool and the digested material was transferred quantitatively to a vacuum jacketed flask of micro Kjeldahl distillation apparatus and the ammonia liberated by the addition of 25ml of 40%NaOH on heating was absorbed in 25ml of boric acid containing 2-3 drops of mixed indicator in 100 ml conical flask. The distilled off ammonia was titrated against 0.1N sulphuric acid. The blank was also run in a similar way.

Calculation

$$\text{Nitrogen (\%)} = \frac{\text{N of } H_2SO_4 \text{x Vol. of 0.1N } H_2SO_4 \text{ x 14}}{\text{Weight of sample x 1000}} \times 100$$

Crude Protein (%) = N % x 6.25/5.83

Fat

The method outlined in AOAC (1998) [1] was used to determine the sample's fat content. The sample (5 g) was precisely weighed, put in a thimble, and covered with cotton. A pre-weighed extraction flask (A) was put on top of the thimble housing the extractor. Soxhlet's extraction method was used to extract the sample using petroleum ether (AR grade 60–80 °C) for 8 hours in order to assess its fat content. Following extraction, any excess solvent was distilled out, and any remaining solvent was destroyed by heating at 80 °C for 4-6 hours in an oven. The flask was weighed (B), and the following formula was used to calculate the fat content.

Calculation

$$\text{Crude fat (\%)} = \frac{\text{Wt. of flask (B)} - \text{Wt. of flask (A)}}{\text{Weight of sample}} \times 100$$

Ash

The method described in AOAC (1998) [1] was used to determine the sample of ash content. The pre-weighed crucible was filled with the sample (5 g). It was entirely charred after being torched on gas flame. The samples were then heated to 520 °C in a muffle furnace for 5 hours of combustion before being cooled in desiccators and weighed. Until a steady weight was achieved, the muffle furnace heating process was repeated. The ash content was determined to be as follows.

Calculation

$$\text{Ash (\%)} = \frac{\text{(Initial wt of empty crucible and sample)} - \text{(Final wt of crucible with ash)}}{\text{Weight of sample}} \times 100$$

Crude fibre

The crude fibre was determined by the method as described in AOAC (1998) [1].

Reagents

1. Sulphuric acid 0.255 N
2. Sodium hydroxide 0.313N

Procedure

Dry defatted sample (2 g) was transferred in to 500ml conical flask to which 200ml of 0.255 N boiling sulphuric acid was added, and then it was boiled for 30 min., kept the volume constant by the addition of water at frequent intervals. The mixture was cooled and filtered through a muslin cloth and the residue was washed with hot water till it was free from acid. The material was then transferred to the same beaker and 200ml of boiling 0.313 N NaOH was added. After boiling for 30 min. the mixture was cooled and again filtered through muslin cloth. The residue was washed with water till it get free from alkali, followed by washing with absolute alcohol and ether to remove the moisture and residue fat. It was then transferred to a weighed crucible and kept in oven at 100 °C for 4-6 hr. The crucible was cooled and weighed. The difference in weight represents the crude fibre content in samples.

$$\text{Crude Fibre (\%)} = \frac{\text{Diff. in wt. of crucible}}{\text{Weight of sample}} \times 100$$

Carbohydrates

The content of carbohydrate in the selected samples were obtained by subtracting from 100, the sum of values of moisture, protein, fat and ash content per 100 g of the sample (Raghuramulu *et al.*, 1993) [10].

Carbohydrate = 100 - (Moisture + Protein + Fat + Ash).

Energy
Food energy value (kcal/100 g) was determined according to the method of Marero *et al.*, (1998) [8] using the factor (4 × % Protein) + (4 × % Carbohydrate) + (9 × % Fat).

Results and discussion
Proximate composition of hulless barley flour cookies
Moisture
The effect of different ingredients and their combination on moisture content of cookies is presented in (Table 2). It ranged from 2.4 to 5.25 percent. The minimum and maximum moisture content in developed cookies was recorded at experiment 17 and 18 respectively. These experiments represented the combination of ingredients as 85:15, 35, 3.5, 3.5 g and 25:75, 35, 3.5, 3.5 g of RWF: HBF, sugar, guar gum, ammonium bicarbonate respectively.
The F-ratio (2.17) is lower than the table value of 2.38, according to the ANOVA table (Table 4). The model's R^2 score is 53.30%, which shows that it is not significant and can only account for 53.30% of the variation in the experiments. The model was therefore deemed insufficient for further research.
At a 5% level of confidence, the probability value of the regression model (Table 3) demonstrates that the amount of barley flour had a positive linear significant effect on the moisture content. Even at a 10% level of confidence, the model's remaining terms were all found to be non-significant.
The cookies' moisture level (10%) was low enough to minimize the risk of microbial deterioration and hence ensure high storage stability (Ayo *et al.*, 2007) [2].

Protein
Cookies made with hulless barley flour ranged in protein level from 11.19 to 14.05 percent (Table 2). At experiments 17 and 18, respectively, the minimum and maximum protein content of cookies were discovered. The proportions of RWF: HBF, sugar, guar gum, and ammonium bicarbonate used in these trials were 85:15, 35, 3.5, 3.5 g and 25:75, 35, 3.5, 3.5 g respectively.
The two factor interaction model's analysis of variance table (Table 4) shows that the model's F-ratio (30.65) was higher than the table value of 2.38 at the 5% level of significance. The model's R^2 value was 94.16 percent. It shows that the model successfully described 94.16 percent of the experiment's variability. Further analysis has been done because the model is deemed adequate.
The analysis of the regression model (Table 3) reveals that the protein content was positively affected by the amount of barley flour at the 5% level of confidence. Even at a 10% level of confidence, the remaining model terms were all judged to be insignificant.
The protein content of the cookies increased significantly (p 0.05) after the addition of hulless barley flour. The substantial amount of protein (12.5%) in barley bran may be responsible for the observed increase (Satinder, Sativa & Nagi, 2011) [11]. The results are consistent with Omeire and Ohambele's (2010) [9] research on the rising protein content trend (8.54-17.72%) in cookies made from wheat-defatted cashew nut flour mixtures.

Fat
Cookies' fat content ranged from 22.39 to 25.25 percent (Table 2). Cookies had a minimum fat content of 17 and a maximum fat content of 18 respectively. In these studies, the ratio of RWF: HBF, sugar, guar gum and ammonium bicarbonate was 85:15, 35, 3.5, 3.5 g and 25:75, 35, 3.5, 3.5 g respectively.
The two factor interaction model's analysis of variance table (Table 4) shows that the model's F-ratio (30.95) was higher than the table value of 2.38 at the 5% level of significance. The model's R^2 value was 94.22 percent, meaning it explained 94.22 percent of the variability in the experiments. Further investigation has been conducted because it is believed that the model is adequate.
Barley flour levels exhibited a positive linear significant effect on fat content at a 5% level of confidence, according to the probability value of the regression model (Table 3). Even at a 10% level of confidence, the other terms in the model were found to be non-significant.
The fat content of the cookies increased significantly ($p < 0.05$) as the substitution level increased from 15 to 75% with hulless barley flour. The finding agrees with Omeire and Ohambele (2010) [9] and Gernah *et al.,* (2010) [4] on their reports.

Ash
According to Table 2, the ash content of cookies varied from 0.83 to 1.61 percent. At experiments 17 and 18, respectively, the lowest and maximum ash content of cookies were discovered. The component combinations in these trials were 85:15, 35, 3.5, 3.5 g of RWF: HBF, sugar, guar gum and ammonium bicarbonate, and 25:75, 35, 3.5, 3.5 g of RWF: HBF, sugar, guar gum respectively.
The whole second order regression model's analysis of variance table (Table 4) showed that the model's F-ratio (1.14) was lower than the table value of 2.38 at the 5% level. Model's R^2 value was 37.50 percent. It shows that a model may account for 37.50 percent of the experimental variability.
Similar findings regarding the high ash content (1.85-2.89%) of cookies made from wheat-brewers spent grain flour blends were also reported by Gernah *et al.*, (2010) [4].

Fibre
The fiber content of the created cookies ranged from 1.6 to 4.45 percent (Table 2). At experiments 17 and 18, respectively, the minimum and maximum fiber content of cookies were discovered. The component combinations in these trials were 85:15, 35, 3.5, 3.5 g of RWF: HBF, sugar, guar gum, and ammonium bicarbonate, and 25:75, 35, 3.5, 3.5 g of RWF: HBF, sugar, guar gum respectively.
The full second order regression model's analysis of variance table (Table 4) shows that the model's F-ratio (2.21) was lower than the table value of 2.38 at the 5% level of significance. This model's R^2 value was 53.74 percent. It shows that the model explained 53.74 percent of the experiment's variability.
The results support the conclusion of Gernah *et al.*, (2010) [4] that cookies manufactured with wheat-brewers waste grain flour blends had an increasing trend in the crude fiber content (1.32-10.82%).

Carbohydrate
The various ingredient combinations and their impact on the amount of carbohydrates in created cookies are listed in (Table 2). The percentage varied from 49.39 to 61.59.

Cookies had a minimum and maximum carbohydrate value of 18 and 17 respectively. The component combinations in these studies were 25:75, 35, 3.5, 3.5 g and 85:15, 35, 3.5, 3.5 g of RWF: HBF, sugar, guar gum, and ammonium bicarbonate respectively.

According to the analysis of variance (table 4), the F-ratio (8.63) is lower than the table value of 2.38. The R^2 value of the perdition model is 81.96%, indicating that it is non-significant and may account for 81.96% of the experimental variability.

The analysis of the regression model (Table 3) demonstrates that the amounts of barley flour had a positive linear significant influence at the 5% level of confidence on the carbohydrate content. Rest of the other model terms were determined to be non-significant even at a 10% level of confidence.

Similar to this, Gernah *et al.*, (2010) [4] and Joel, Fatima, and Stephen (2014) [7] found declining trends in the carbohydrate contents (73.46 - 46.20%) and (70.45-23.71%) of cookies manufactured from wheat-brewers waste grain flour blends and whole wheat-full fat soya flour blends respectively.

Energy

Cookies provided an energy value between 479.02 and 493.84 kcal (Table 2). The lowest and highest energy contents of the cookies were represented by Experiment 27 and Experiment 4. The ingredient combinations in these studies were 55:45, 35, 3.5, 3.5 g and 40:60, 40, 2.5, 3 g of RWF: HBF, sugar, guar gum, and ammonium bicarbonate, respectively.

Giwa and Ikujenlola (2010) observed a declining trend in the energy value (443.89-431.95 kcal) for cookies prepared from wheat and high-quality protein maize.

Table 2: Chemical composition of developed hulless barley flour and refined wheat flour cookies

Exp. Unit	**Moisture %**	**Protein %**	**Fat %**	**Ash %**	**Fiber %**	**Carbohydrate %**	**Energy (K Cal)**
1	3.12	11.6	22.8	0.99	2.32	59.17	488.28
2	3.76	13.64	24.84	1.11	2.96	53.69	492.88
3	3.2	11.6	22.8	0.95	2.4	59.05	487.8
4	3.65	13.64	24.84	1.09	2.85	53.93	493.84
5	3.02	12.01	23.21	0.92	2.22	58.62	491.41
6	3.88	13.23	24.43	1.07	3.08	54.31	490.03
7	3.21	12.01	23.21	1	2.41	58.16	489.57
8	3.92	13.23	24.43	1.13	3.12	54.17	489.47
9	2.98	12.01	23.21	0.89	2.18	58.73	491.85
10	3.84	13.23	24.43	1.21	3.04	54.25	489.79
11	3.32	11.6	22.8	0.91	2.52	58.85	487
12	3.92	13.23	24.43	1.23	3.12	54.07	489.07
13	3.22	11.6	22.8	0.91	2.42	59.05	487.8
14	4.01	13.23	24.43	1.08	3.21	54.04	488.95
15	2.87	11.6	22.8	1.09	2.07	59.57	489.88
16	4.12	13.23	24.43	1.13	3.32	53.77	487.87
17	2.4	11.19	22.39	0.83	1.6	61.59	492.63
18	5.25	14.05	25.25	1.61	4.45	49.39	481.01
19	4.24	12.42	23.62	1.23	3.44	55.05	482.46
20	4.41	12.42	23.62	1.45	3.61	54.49	480.22
21	4.66	12.83	24.03	1.11	3.86	53.51	481.63
22	4.14	12.42	23.62	1.29	3.34	55.19	483.02
23	4.03	12.83	24.03	1.41	3.23	54.47	485.47
24	4.21	12.83	24.03	1.32	3.41	54.2	484.39
25	4.2	12.83	24.03	1.21	3.4	54.33	484.91
26	3.98	12.42	23.62	1.15	3.18	55.65	484.86
27	4.54	12.42	23.62	1.49	3.74	54.19	479.02
28	4.71	12.83	24.03	1.37	3.91	53.15	480.19
29	3.94	12.42	23.62	1.51	3.14	55.37	483.74
30	4.42	12.83	24.03	1.3	3.62	53.8	482.79

Table 3: Regression coefficient of two factor interaction model and significant term for proximate composition of hulless barley cookies

Coefficient	**Moisture**	**Protein**	**Fat**	**Ash**	**Fiber**	**Carbohydrate**
Intercept	1.95	3.55	4.88	1.08	1.73	7.45
Linear						
β1 A	0.1306	0.1079*	0.0784*	0.0582	0.1487	**-0.1764***
β2 B	0.0076	-0.0025	-0.0018	0.0151	0.0086	-0.0040
β3 C	-0.0059	-0.0070	-0.0052	0.0061	-0.0066	0.0094
β4 D	0.0089	-0.0071	-0.0052	0.0002	0.0098	0.0018
Interactive						
β 1.2 AB	-0.0027	0.0037	0.0027	-0.0043	-0.0031	-0.0017
β 1.3 AC	0.0174	-0.0107	-0.0078	-0.0123	0.0198	0.0031
β 1.4 AD	0.0138	-0.0033	-0.0025	0.0094	0.0156	-0.0048
β2.3 BC	-0.0074	0.0037	0.0027	0.0121	-0.0088	-0.0018
β2.4 BD	-0.0009	-0.0037	-0.0027	0.0059	-0.0013	0.0026
β 3.4 CD	-0.0026	-0.0042	-0.0029	0.0003	-0.0030	0.0043

**Significant at 1%, *Significant at 5%, β1-Hulless Barley flour, β2-Sugar, β3-Guar gum, β4-Ammonium bicarbonate.

Table 4: ANOVA of two factor interaction model for proximate composition of hulless barley cookies

Source	Moisture	Protein	Fat	Ash	Fiber	Carbohydrate
Model SS	0.4223	0.2852	0.1506	0.0948	0.5478	0.7504
Model MS	0.0422	0.0285	0.0151	0.0095	0.0548	0.0750
Model DF	10	10	10	10	10	10
Error SS	0.0280	0.0050	0.0026	0.0201	0.0345	0.0205
Error MS	0.0056	0.0010	0.0005	0.0040	0.0069	0.0041
Error DF	5	5	5	5	5	5
F Ratio	2.17	30.65	30.95	1.14	2.21	8.63
F Table	2.38	2.38	2.38	2.38	2.38	2.38
R Square	0.5330	0.9416	0.9422	0.3750	0.5374	0.8196
Std dev.	0.1395	0.0305	0.0221	0.0912	0.1575	0.0932
Mean	1.95	3.55	4.88	1.08	1.73	7.45
C.V.	7.15	0.8603	0.4524	8.48	9.09	1.25

MS: Mean square; SS: Sum of squares; DF: Degree of freedom; Std. dev.: Standard deviation

Conclusion

According to estimates, the bakery industry's share of biscuits and cookies is about 37% by volume and 75% by value. Due to the range of the moisture, fat, protein, fiber, carbohydrate and ash content were found to be 12.3, 1.3, 10.1, 1.2, 75.52, 0.78 percent in refined wheat flour and 10.01, 2.2, 11.5, 1.5, 73.9, 0.89 percent in hulless barley flour respectively.

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