

# Development of High Protein Composite flour pre-mix for Women Using Response Surface Methodology

## Research Article

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**Article Information:** Submission: 01/03/2022; Accepted: 08/04/2022; Published: 11/04/2022

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### Abstract

**Background:** India has a higher ratio of malnourished women in the world. Cereals and pulses are said to be the best combination for delivering good nutrients, particularly in the dietary pattern of low economic populations.

**Objectives:** The present investigation is the development of a nutritious composite flour mix for women that can be cooked into a healthy porridge in water or skimmed milk to meet her daily nutrient requirements as per the recommendation of the Indian Council of Medical Research (ICMR), 2020.

**Methods:** The tool used was the Central Composite Rotatable Design of Response Surface Methodology. Locally available and nutrient-rich cereals, pulses and oilseed were selected based on preliminary trials. The sensory responses for color and appearance, body and texture, taste, odor and overall acceptability were studied and statistical validation was done using SPSS 22 software.

**Results:** Maximum sensory scores were obtained for an ingredient composition of 65.95, 37.50 and 3.99 grams of cereal blend, pulse blend and oilseed respectively. The proximate analysis of the optimized product gave 17.06%, 5.21%, 3.67%, 2.96%, 65.09%, and 1.13% of protein, fat, crude fiber, ash, carbohydrate, and calcium. 100g of the optimized product could meet 17.63% of energy and 100% of the calcium requirements for women as per RDA recommendation.

**Keywords:** Cereal and pulse blend; Composite flour; High protein pre-mix; Women health mix

### Introduction

In India, the rate of malnutrition among adolescent girls, pregnant and lactating women shows high trend as per UNICEF and WHO [1]. The diet of Indian women does not meet their nutritional requirements. Addressing women's malnutrition brings forth healthy women who play multiple roles in society which helps them raise the socio-economic status of the country. The ICMR – National Institute of Nutrition (NIN) Expert Group suggestion in 2020, on Nutrient Requirement for Indians reported that women in moderate category of work need 2130 kilocalories of energy per day, which includes 36g protein and 25g visible fat. Females of age between 19 and 39 years are grouped under the category “women” with an average body weight of 55kg [2].

A survey conducted by the Associated Chamber of Commerce and Industry in India reveals that 68% of working women are affected with lifestyle ailments like obesity, chronic backache, diabetes, hypertension and 75% of them suffer from depression or general anxiety disorder [3]. A healthy diet and adequate physical activity can mitigate the situation. Intake of dietary fiber can reduce the risk of type II diabetes and cardiovascular disease and improves serum lipid levels, and lowers blood pressure [4-7]. *In vitro*, animal and human *in vivo* studies on the health benefits of cereal grains found that a calorie restricted whole grain wheat diet improves body composition and reduce fat mass percentage in overweight women [8]. Work carried out by Kaur et al., (2011) revealed association between the legume consumption and declined prevalence of cancer, diabetes and cardiovascular diseases [9]. Isoflavone compounds especially

genistein found in soyabean can be very beneficial to diabetic patients particularly those suffering from Non-Insulin Dependent Diabetes Mellitus [10].

Cereal grains are the fruit of plants belonging to the grass family Gramineae. They are good source of protein, minerals like calcium, iron, phosphorus, zinc, vitamins of group B and contain amino acid [11]. Wheat is the main cereal crop in India next to rice. Barley is ranked fourth among grains in quantity. Millets are the powerhouse of nutrition. Finger millet and foxtail millet are a common variety of grains. They are high in phytochemicals such as polyphenols and dietary fiber [12]. They are also proven to be anti-diabetic, anti-tumorigenic, arteriosclerogenic, antioxidant and antimicrobial [13].

Legumes rich in proteins are taken by deprived sections of society. Peanuts, soya beans and lentils are common in human diets. Legumes are good sources of fiber, gives protection against diabetes and coronary heart diseases and help in weight control [14]. Soybean is rich in vitamins like thiamin, riboflavin, niacin, pantothenic acid, folic acid, A, D, E and K [15]. Sesame seed/oil is a perfect complement to the daily vegan diet. Soy, rapeseed, sunflower, coconut, olive, peanut and flax seeds are also commonly used oils. Hsu and Parthasarathy (2017) reported that legumes help in the reduction of blood lipids and cholesterol in the plasma lowers blood pressure and act neuron protectively [16].

Whole cereals and legumes complement each other. Cereal grains are deficient in certain essential amino acids, especially lysine [17]. Legumes, on the other hand are rich in lysine, but poor in methionine [18]. A clever selection of the ingredients will help to deliver good nutrition to the public [19,20]. Composite flour technology is the process of mixing wheat flour with cereals or legumes to use the locally available raw materials for the production of high-quality food products economically. Today it is being introduced by emerging food industries and health professionals to alleviate the deep-rooted health problems in society and has become a subject of many research studies. Noor Aziah et al., (2012) reported that the incorporation of chickpea flour and mung bean flour in wheat flour increases protein and resistant starch content and also found to improve the acceptability of composite flour cookies [21]. Composite flour bread using refined wheat flour, sprouted mung bean flour, soy flour and mango kernel flour was found to give good organoleptic and physical properties [22]. The impact of kodo and barnyard millet flour blend in the composite mix containing whole wheat flour and defatted soy flour was studied [23]. The physico-chemical and functional properties of the resultant flour blend was found to increase significantly with increased level of incorporation of millet flour blend.

There is no available report on the development of a composite flour mix for women utilizing these nine (wheat, barley, foxtail millet, finger millet, green gram, horse gram, green peas, soyabean and black sesame) locally available nutrient-rich ingredients. Keeping in mind the customer's demand for healthier food with high sensory quality, the present investigation was undertaken using RSM as a statistical approach for finding a relationship between several independent variables and five response variables. Central Composite Rotational Design (CCRD) is one of the design that can estimate a second-degree polynomial model, which can be used to optimize (maximize, minimize, or attain a specific target for) the response variable(s) of interest and has successfully been applied for the development and

optimization of cereal products [24,25]. Thus, in the present study, an attempt is made to formulate and optimize a composite flour mix for women based on the guidelines of ICMR 2020 [26].

## Materials & Methods

The investigation was conducted at the Department of Dairy Technology, Verghese Kurien Institute of Dairy and Food Technology, Thrissur, Kerala during the period 2021- 2022. The ingredients selected for the study were wheat (*Triticum aestivum*), barley (*Hordeum vulgare*), finger millet (*Eleusine coracana*), foxtail millet (*Setaria italica*), green gram (*Vigna radiata*), horse gram (*Macrotyloma uniflorum*), green peas (seed of the pod fruit *Pisum sativum*), soybean (*Glycine max*) and black sesame seed (*Sesamum indicum*). Good quality items were purchased from the local market. They were cleaned, washed, dried, partially heat roasted (5 minutes), grounded and sieved to obtain respective flours which were stored in airtight containers until use. The ingredients for the study were selected in such a way that of the total calories obtained, 60 parts should be met from cereals, 35 parts from pulses and 5 parts from the oilseed. Sensory evaluation was done by seven semi-trained judges (the majority were working women). In preliminary trials, the best cereal blend and best pulse blend were selected (Table 1 and 2). Green gram was randomly used (to represent the pulse component) in the former to select the best cereal blend, which in turn was used (as cereal component) in the latter to select the best pulse blend. Porridge was prepared uniformly for all treatments and was evaluated by seven semi-trained judges for color and appearance, body and texture, taste, odor and overall acceptability of the product. All measurements were taken in triplicates under the same conditions. Kruskal-Wallis test for independent samples was done at a significance level of 5 percent for statistical analysis.

## Experimental Design

RSM models to optimize the relationship among the cereal blend, pulse blend and oil seed at different levels of addition on the sensory properties are outlined here. The software used was Design Expert® 9.0 version of Stat-Ease, Inc, 2021E, Hennepin Avenue, Minneapolis,

**Table 1:** Experimental model for the selection of best cereal blend.

Ingredients/Treatments	T <sub>1</sub> (g)	T <sub>2</sub> (g)	T <sub>3</sub> (g)	T <sub>4</sub> (g)	T <sub>5</sub> (g)
Wheat	15	0	20	20	20
Barley	15	20	0	20	20
Finger millet	15	20	20	0	20
Foxtail millet	15	20	20	20	0
Green gram	35	35	35	35	35
Sesame seed	5	5	5	5	5
	100	100	100	100	100

**Table 2:** Experimental model for the selection of best pulse blend.

Ingredients/Treatments	T <sub>6</sub> (g)	T <sub>7</sub> (g)	T <sub>8</sub> (g)	T <sub>9</sub> (g)	T <sub>10</sub> (g)
Green gram	8.75	0	11.67	11.67	11.67
Horse gram	8.75	11.67	0	11.67	11.67
Green peas	8.75	11.67	11.67	0	11.67
Soybean	8.75	11.67	11.67	11.67	0
Best cereal blend (as determined)	60	60	60	60	60
Sesame seed	5	5	5	5	5
	100	100	100	100	100

USA. The second-order CCRD of these three independent factors suggested 20 trials which were performed taking sensory scores as responses (Table 1). The responses were again fed to the software to optimize the proportion of the factors studied. The minimum and maximum levels of the variables and the design matrixes are depicted in Tables 3 and 4. The basic model equation to fit the data is given by the equation:

$$Y = \beta_0 + \sum_{i=0}^n \beta_i X_i + \sum_{i=0}^n \beta_{ii} X_i^2 + \sum_{i \neq j=1}^n \beta_{ij} X_i X_j \quad (\text{Equation 1})$$

Where Y is the predicted response,  $\beta_0$  is the intercept, n is the number of variables studied,  $\beta_i$ ,  $\beta_{ii}$  and  $\beta_{ij}$  are the linear (effect of that single factor), quadratic (square terms) and interactive model coefficients,  $X_i$  and  $X_j$  represents the levels of the independent parameters. Positive or negative coefficients indicate synergistic or antagonistic effect of that factor on the observed response. The predicted values of the sensory responses corresponding to the optimum levels of the factors were compared with the actual responses using a one-sample t-test. 3D surface graphs were used to observe the response variable at its optimal level.

### Characterization of the Optimized Composite Flour

#### A. Proximate analysis and energy value

The moisture, protein, carbohydrate, fat, crude fiber and ash

**Table 3:** Levels of independent variables for the experimental design.

Independent variable	Minimum	Maximum
	(Parts of the total mix)	
A: Cereal Blend	50	70
B: Pulse Blend	35	40
C: Oil Seed	0	5

**Table 4:** Design matrix used for the optimization of the composite flour.

Stdorder	Factors		
	A: Cereal Blend	B: Pulse Blend	C: Oil Seed
1	54.05	36.01	1.01
2	65.95	36.01	1.01
3	54.05	38.99	1.01
4	65.95	38.99	1.01
5	54.05	36.01	3.99
6	65.95	36.01	3.99
7	54.05	38.99	3.99
8	65.95	38.99	3.99
9	50.00	37.50	2.50
10	70.00	37.50	2.50
11	60.00	35.00	2.50
12	60.00	40.00	2.50
13	60.00	37.50	0.00
14	60.00	37.50	5.00
15	60.00	37.50	2.50
16	60.00	37.50	2.50
17	60.00	37.50	2.50
18	60.00	37.50	2.50
19	60.00	37.50	2.50
20	60.00	37.50	2.50

content of the composite flour were analyzed as per Association of Official Analytical Collaboration (AOAC), International 2016 [27]. The total energy provided by the flour was estimated by summing up the number of carbohydrates, protein, and fat which were multiplied by the respective conversion factors such as 4, 4, and 9 kcal/g [28]. The per cent daily value of the components was calculated based on the recommendation of ICMR 2020 for Indian woman.

#### B. Physical properties

**Colour:** The color of the flour sample was observed by hunter lab digital colorimeter (model D25L-9, Hunter Associates Lab, Inc.). Composite flour was scanned at three different locations and the hunter values  $L^*$ ,  $a^*$ , and  $b^*$  were recorded. A low number (0–50) of  $L^*$  value indicates dark and a high number (51–100) indicates light. A positive number of  $a^*$  value indicates red and a negative number indicates green. Similarly, a positive number of  $b^*$  value indicates yellow and a negative number indicates blue. Hue angle was determined from the formula  $^{\circ}Hue = \tan^{-1} \frac{b^*}{a^*}$ . It determines the darkness or lightness of the shade.

**Bulk density:** Bulk density was measured using standard procedures as done by Jones et al., 2000 using a Tapped Density meter calibrated with 250 drops/ min [29].

$$\text{Bulk density (g/ml)} = \frac{\text{weight of sample}}{\text{volume of sample after tapping}}$$

#### Water Absorption Capacity (WAC)

The Water Absorption Capacity of the composite flour was studied as per the work of Duguma et al., 2021 [30]. One gram of the composite flour was taken in a 50 ml centrifuge tube and added with 10 ml of distilled water which was thoroughly mixed in a vortex mixer for 3 minutes and centrifuged at 3500 rpm for 30 minutes. The water absorbed by the flour as percent water-bound per gram of flour was determined as below.

$$WAC = \frac{(W_3 - W_2)}{W_1}$$

Where  $W_1$  = weight of flour taken,  $W_2$  = weight of the empty tube,  $W_3$  = weight of flour plus tube after centrifuge

### Results & Discussion

Sensory attributes are the primary factor that needs to be considered for the development of any new product. Preliminary trials were conducted to select the best cereal blend (from among the cereals) and the best pulse blend (from among the pulses) considering a fixed amount of the oil seed, in the proportion of 60 parts of cereals, 35 parts of pulses and 5 parts of oil seed in the final blend. Four cereals, four pulses and an oil seed were used for the study. 9-point hedonic scale was used for the preliminary evaluation. Table 1 and 2 shows the components of the ten treatments (five each of best cereal blend and of best pulse blend). Three replications were done to study statistical significance. Table 5 shows the sensory scores of all treatments for the selection of best cereal blend.  $T_1$  received highest score for colour and appearance ( $8.00 \pm 0.001$ ), body and texture ( $8.33 \pm 0.67$ ), taste ( $8.33 \pm 0.67$ ), odour ( $8.00 \pm 0.67$ ) and overall acceptability ( $8.33 \pm 0.33$ ) and was selected as the best cereal blend. Table 6 shows the sensory

scores of all treatments for the selection of best pulse blend.  $T_6$  with all the pulses had the values of  $8.00 \pm 0.88$ ,  $7.83 \pm 0.75$ ,  $8.08 \pm 0.92$ ,  $8.08 \pm 1.00$  and  $8.17 \pm 0.96$  for the sensory responses. No significant difference was observed among the various treatments and no cereal or pulse was found to give any unacceptable sensory response. Hence  $T_1$  and  $T_6$  were selected and all the cereals in equal proportion was regarded as the best cereal blend and all the pulses in equal proportion was considered as the best pulse blend for further study.

### Optimization Using RSM

The proportion of the cereal blends (A), pulse blend (B) and oilseed (C) in the final composite flour was optimized using CCRD of RSM. Table 7 and 8 represent the suggested experimental design and mean values of sensory attributes and the regression equations for the responses. The various responses studied were colour and appearance, body and texture, taste, odour and overall acceptability. Equation (1) fit the obtained results well and was statistically significant at different p values. The adequacy of quadratic models was confirmed by the Analysis of Variance (ANOVA) using Fisher's test value (F-value) and lack-of-fit test.

### Fitting and Validating RSM models for optimizing independent variables

Table 7 and Table 8 show the linear, interactive and quadratic effects of each independent parameter on the observed response. The final predictive equation neglecting the non-significant terms are given below. In all the equations, the coefficient of determination ( $R^2$ ) and adequate precision value (APV) were at desirable levels.

**Table 5:** Selection of the best cereal blend.

Attributes/ Treatments	Colour and Appearance	Body and Texture	Flavour		Overall Acceptability
			Taste	Odour	
	Median $\pm$ Interquartile range				
T <sub>1</sub>	8.00 $\pm$ 0.001	8.33 $\pm$ 0.67	8.33 $\pm$ 0.67	8.00 $\pm$ 0.67	8.33 $\pm$ 0.33
T <sub>2</sub>	8.00 $\pm$ 0.33	7.67 $\pm$ 0.67	7.67 $\pm$ 0.83	8.00 $\pm$ 0.33	7.67 $\pm$ 0.67
T <sub>3</sub>	8.00 $\pm$ 0.67	8.00 $\pm$ 1.00	7.67 $\pm$ 1.00	7.67 $\pm$ 0.33	7.67 $\pm$ 0.50
T <sub>4</sub>	8.00 $\pm$ 0.67	7.83 $\pm$ 1.50	7.33 $\pm$ 1.67	8.00 $\pm$ 1.00	8.00 $\pm$ 1.67
T <sub>5</sub>	7.67 $\pm$ 0.67	7.33 $\pm$ 0.83	7.50 $\pm$ 0.67	7.67 $\pm$ 1.00	7.50 $\pm$ 0.83
$\lambda^2$ value	5.05	8.18	9.16	4.30	7.14
p-value	0.28 <sup>ns</sup>	0.09 <sup>ns</sup>	0.06 <sup>ns</sup>	0.37 <sup>ns</sup>	0.13 <sup>ns</sup>

All observations are done in three replications by seven semi-trained judges. ns- non-significant ( $p \geq 0.05$ )

**Table 6:** Selection of best pulse blend.

Attributes/ Treatments	Colour and Appearance	Body and Texture	Flavour		Overall Acceptability
			Taste	Odour	
	Median ± Interquartile range				
T <sub>6</sub>	8.00±0.88	7.83±0.75	8.08±0.92	8.08±1.00	8.17±0.96
T <sub>7</sub>	7.50±0.67	7.60±0.83	7.33±0.67	7.67±0.67	7.67±0.33
T <sub>8</sub>	7.83±1.33	7.83±0.83	8.00±0.50	7.50±1.00	8.33±0.67
T <sub>9</sub>	7.67±0.50	7.67±1.00	7.50±0.83	7.67±1.00	7.83±0.67
T <sub>10</sub>	7.67±0.50	8.00±1.00	7.67±0.67	7.67±0.50	7.83±0.50
λ <sup>2</sup> value	2.95	2.50	8.54	1.63	5.52
p-value	0.57 <sup>ns</sup>	0.64 <sup>ns</sup>	0.07 <sup>ns</sup>	0.80 <sup>ns</sup>	0.24 <sup>ns</sup>

All observations are done in three replications by seven semi-trained judges. ns- non-significant ( $p \geq 0.05$ )

**Table 7:** Mean values for the sensory responses for the different combinations of flour.

Std Run	Responses				
	Colour and Appearance	Body and Texture	Taste	Odour	Overall acceptability
1	7.78	7.67	8.00	7.92	7.92
2	8.08	7.83	7.92	7.96	8.33
3	7.83	7.92	8.08	8.00	7.83
4	8.00	8.17	7.83	7.92	7.92
5	7.88	7.96	7.96	7.96	8.00
6	8.25	8.00	8.08	8.08	8.33
7	7.75	7.92	7.96	7.96	8.00
8	8.00	7.92	7.92	7.96	7.92
9	7.67	7.58	7.92	8.08	7.83
10	8.08	7.67	7.83	8.08	8.08
11	8.08	8.17	8.08	7.96	8.33
12	7.83	8.33	8.00	7.92	8.00
13	8.00	7.83	7.92	7.83	7.92
14	8.04	8.00	7.97	7.92	8.00
15	7.92	8.17	7.92	7.96	8.08
16	7.92	8.08	7.92	7.96	8.08
17	7.92	8.08	7.92	7.96	8.00
18	7.92	8.08	7.92	8.00	8.08
19	8.00	8.00	7.96	7.96	8.00
20	7.92	7.92	7.92	7.96	8.08

**Table 8:** Regression coefficients and ANOVA fitted quadratic models for all the responses.

Coefficients	Sensory Responses				
	Colour and Appearance	Body and Texture	Taste	Odour	Overall acceptability
Intercept	7.93	8.05	7.93	7.97	8.05
<b>Linear</b>					
A-Cereal Blend	0.13 <sup>**</sup>	0.04 <sup>*</sup>	-0.03 <sup>**</sup>	0.01 <sup>ns</sup>	0.09 <sup>**</sup>
B-Pulse Blend	-0.06 <sup>**</sup>	0.05 <sup>*</sup>	-0.02 <sup>**</sup>	-0.01 <sup>*</sup>	-0.11 <sup>**</sup>
C-Oil Seed	0.02 <sup>*</sup>	0.04 <sup>ns</sup>	0.01 <sup>**</sup>	0.02 <sup>**</sup>	0.03 <sup>*</sup>
<b>Interaction</b>					
AB	-0.03 <sup>*</sup>	0.01 <sup>ns</sup>	-0.04 <sup>**</sup>	-0.03 <sup>**</sup>	-0.09 <sup>**</sup>
AC	0.02 <sup>ns</sup>	-0.05 <sup>ns</sup>	0.05 <sup>**</sup>	0.02 <sup>**</sup>	-0.03 <sup>*</sup>
BC	-0.04 <sup>**</sup>	-0.09 <sup>**</sup>	-0.02 <sup>**</sup>	-0.02 <sup>**</sup>	0.01 <sup>ns</sup>
<b>Quadratic</b>					
A <sup>2</sup>	-0.02 <sup>*</sup>	-0.15 <sup>**</sup>	-0.02 <sup>**</sup>	0.04 <sup>**</sup>	-0.03 <sup>**</sup>
B <sup>2</sup>	0.01 <sup>*</sup>	0.07 <sup>**</sup>	0.04 <sup>**</sup>	-0.01 <sup>*</sup>	0.04 <sup>**</sup>
C <sup>2</sup>	0.03 <sup>**</sup>	-0.05 <sup>*</sup>	0.01 <sup>*</sup>	-0.03 <sup>**</sup>	-0.03 <sup>**</sup>
<b>Model Fit Statistics</b>					
Lack-of-fit	0.73 <sup>ns</sup>	0.95 <sup>ns</sup>	0.73 <sup>ns</sup>	0.81 <sup>ns</sup>	0.83 <sup>ns</sup>
F-value	44.69 <sup>*</sup>	15.90 <sup>*</sup>	48.57 <sup>*</sup>	38.76 <sup>*</sup>	37.63 <sup>*</sup>
R <sup>2</sup>	0.98	0.93	0.98	0.97	0.97
PRESS value	0.03	0.11	0.01	0.01	0.04
Adequate Precision	29.33	16.90	25.49	25.90	21.90

<sup>\*\*</sup> - Highly significant ( $p < 0.01$ ), <sup>\*</sup> - Significant ( $p < 0.05$ ), ns- non-significant ( $p > 0.05$ ).



**Colour and appearance** =  $7.93 + 0.13A - 0.06B + 0.02C - 0.03AB - 0.04BC - 0.02A^2 + 0.01B^2 + 0.03$  ( $R^2 = 0.98$ ,  $APV = 29.33$ ) (Equation 2)

**Body and Texture** =  $8.05 + 0.04A + 0.05B - 0.09BC - 0.15A^2 + 0.07B^2 - 0.05C^2$  ( $R^2 = 0.93$ ,  $APV = 16.90$ ) (Equation 3)

**Taste** =  $7.93 - 0.03A - 0.02B + 0.01C - 0.04AB + 0.05AC - 0.02BC - 0.02A^2 + 0.04B^2 + 0.01C^2$  ( $R^2 = 0.98$ ,  $APV = 25.49$ ) (Equation 4)

**Odour** =  $7.97 - 0.01B + 0.02C - 0.03AB + 0.02AC - 0.02BC + 0.04A^2 - 0.01B^2 - 0.03C^2$  ( $R^2 = 0.97$ ,  $APV = 25.90$ ) (Equation 5)

**Overall acceptability** =  $8.05 + 0.09A - 0.11B + 0.03C - 0.09AB - 0.0312 + 0.01BC - 0.03A^2 + 0.04B^2 - 0.03C^2$  ( $R^2 = 0.97$ ,  $APV = 21.90$ ) (Equation 6)

It is observed that cereal blend (A) positively affected colour and appearance, body and texture and overall acceptability at linear terms, while negatively affected taste (equation 4). This may be due to the individual cereals especially finger millet and foxtail millet which impart typical taste. Pulse blend had a positive effect on body and texture, but negatively influenced its colour and appearance, taste, odour and overall acceptability. Typical beany flavour and colour of soybean, green gram and horse gram might have contributed to the effect. It is in accordance with the findings of Yadav et al., 2021 and Kadam et al., 2012 [31,32]. Increase in the proportion of oilseed significantly improved ( $p < 0.05$ ) colour and appearance, taste, odour and overall acceptability, while influenced body and texture non-significantly ( $p > 0.05$ ) (equation 3). This may be due to the granular texture of sesame seed.

Studying the interaction behaviour of A and B at a constant level of C, it was found that an inverse relationship exists in colour and appearance and a similar effect was found for other responses like odour, taste and overall acceptability (Figure 1A,G,J,M), which were significant ( $p < 0.01$ ). With respect to body and texture, a positive correlation was observed between the two variables (Fig. 1D), but the impact was not statistically significant [31, 32]. The interaction effects of the cereal blend and oilseed at a constant level of pulse blend, on the various independent responses are depicted in Figure 1B,E,H,K and N. A significant positive effect ( $p < 0.01$ ) was observed on taste and odour of the product as depicted in Figure 1H and K. The overall acceptability was improved initially with the increase in cereal blend, but tends to decrease significantly with an increase in oilseed as showed in Figure 1N and showed non-significant influence on the other responses. The interaction effect of pulse blend and oilseed are depicted in Figure 1C,F,I,L and O. They showed a significantly negative correlation for colour and appearance, body and texture, taste and odour. The proportion of oilseed improved the sensory response, but the pulse blend showed antagonistic effect. Though they showed a positive interaction effect on overall acceptability, it was not statistically significant. The beany flavour and hygroscopicity of the pulses might have attributed to the effect.

The square increase in the amount of cereal blend (A) was found to improve the odour of the product, which may be due to the pleasing flavour of barley and finger millet, while the other sensory responses had a significantly negative impact. The effects of pulse blend (B) and oilseed (C) are represented in Table 8. The maximum sensory score for colour and appearance, body and texture, taste, odour and overall

acceptability were 8.08, 8.17, 8.08, 8.08 and 8.33 respectively (Table 7). The composite flour mix was thus optimized by superimposing the sensory scores at their maximum levels.

### Statistical Verification of the Predicted Value

Fitted predicted models had coefficients of 8.25, 7.99, 8.08, 8.08, 8.30 respectively for colour and appearance, body and texture, taste, odour and overall acceptability of the final product corresponding to the optimized composition of 65.95 gram of cereal blend, 36.01 gram of pulse blend and 3.98 grams of oilseed. These predicted sensory scores were verified by preparing the product corresponding to the suggested optimum values of the independent variables and performing sensory evaluation on 9-point hedonic scale. The experiment was performed four times to validate the predicted value. Table 9 shows the mean observed sensory scores with corresponding t values. It was found that no significant difference exists between the predicted and observed values. Thus, the level of cereal blend, pulse blend and oilseed were confirmed.

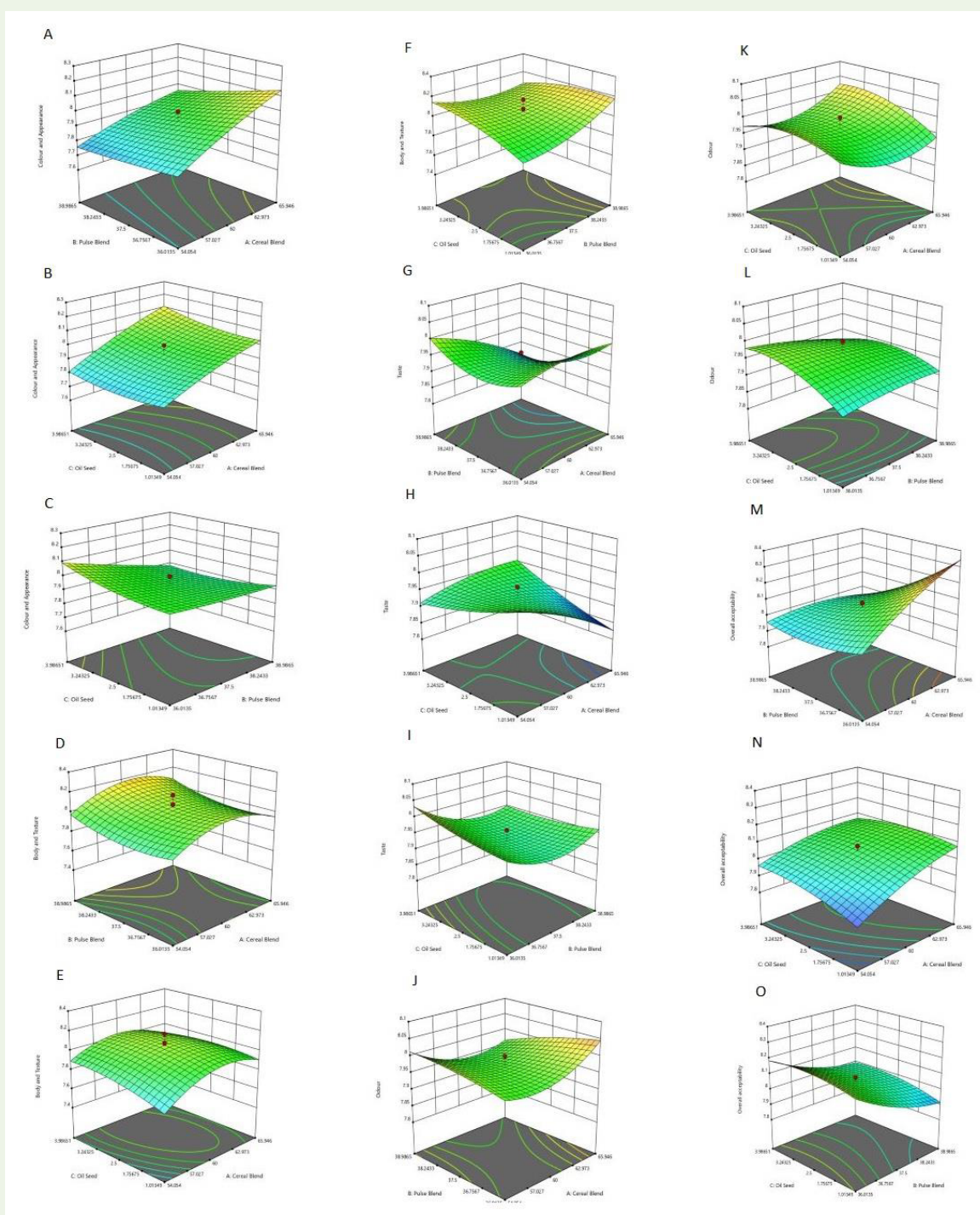
### Characterization of the Composite Flour

#### A. Proximate Composition

The composite flour optimized by sensory responses was then evaluated for its nutritive and physico-chemical properties. The proximate composition of the optimized flour is depicted in Table 10. The low moisture content of 6.01 % was observed. It retards deteriorations like lipolysis, proteolysis and microbial growth [33]. Kumar et al., 2015 reported a moisture content of 13% in multigrain flour containing wheat, sorghum, barley, chickpea and pea flour in equal proportions [34]. Tharise et al., 2014 formulated a composite flour using soyabean with cereals, which was found to have 9.37% to 12.07% moisture [35]. All the flour components especially pulses like soyabean are rich in protein. The composite flour had 17.06% protein on a dry basis. The result is approximately in accordance with Poongodi and Mohankumar, 2009 [36], who obtained high protein for their various millet- pulse-based composite flour. A high protein diet supplies essential amino acids for tissue repair and does not raise blood glucose during absorption. ICMR recommends a daily intake of 46g of protein for a woman. 100g of the formulated flour can supply about 37% of the daily value of protein. 5.21% of fat on a dry basis reflects the fat in the individual components, which are natural fat. It is in nearly in agreement with the observed readings of Mounika and Hymavathi (2021) [37]. Fat is essential for energy and delays gastric emptying leading to a decreased glycemic response to a meal [38]. The fiber content of 3.67% can be attributed to the fiber-rich millet grains. It helps in a lower rate of weight gain in women. A mineral composition of 2.96% was observed in the flour with 1.13% calcium

**Table 9:** Predicted values vs observed values of the experimental responses.

Attributes		Predicted value	Observed value	t-value	p-value
		Mean± SE			
Colour and Appearance		8.25±0.02	8.48±0.17	1.37	0.22 <sup>ns</sup>
Body and Texture		7.99±0.05	8.34±0.19	1.84	0.12 <sup>ns</sup>
Flavour	Taste	8.08±0.01	8.14±0.15	0.43	0.68 <sup>ns</sup>
	Odour	8.08±0.01	8.34±0.23	1.15	0.30 <sup>ns</sup>
Overall Acceptability		8.30±0.03	8.50±0.21	0.97	0.37 <sup>ns</sup>



**Figure 1:** The response surface plots of the sensory responses as affected by the independent variables

A: Effect of cereal blend and pulse blend on colour and appearance B: Effect of cereal blend and oil seed on colour and appearance C: Effect of pulse blend and oil seed on colour and appearance D: Effect of cereal blend and pulse blend on body and texture E: Effect of cereal blend and oil seed on body and texture F: Effect of pulse blend and oil seed on body and texture G: Effect of cereal blend and pulse blend on taste H: Effect of cereal blend and oil seed on taste I: Effect of pulse blend and oil seed on taste J: Effect of cereal blend and pulse blend on odour K: Effect of cereal blend and oil seed on odour L: Effect of pulse blend and oil seed on odour M: Effect of cereal blend and pulse blend on overall acceptability N: Effect of cereal blend and oil seed on overall acceptability. O: Effect of pulse blend and oil seed on overall acceptability.

and 0.72% phosphorus. The optimum calcium to phosphorus ratio will favour its maximum absorption in the body. Karuppaswamy et al., 2013 also observed a higher mineral content in composite flour added with millets [39]. Carbohydrate level in cereals is high when compared to pulses; hence a clever selection of ingredients helps to give good nutrients at lower carbohydrate levels. Similar results were reported where cereal-pulse-based composite flour contained 67% of carbohydrate [37]. The process of roasting the grains will improve the digestibility of the flour by reducing its anti-nutritional factors [40]. The flour had a high energy value (375.43 KCal/100g). This is higher than reported by others [37], Indian women need 2130 kilocalories of energy per day (ICMR 2020). 100g of the composite flour developed in the study can supply 17.6 percent of the daily requirement (Table 10).

### B. Physical parameters

The colour, bulk density and water absorption capacity of the optimized flour is depicted in Table 11. The perception of colour influences the acceptability of the product. The positive values of  $a^*$  and  $b^*$  shows that it has a reddish yellow shade. This may be contributed by finger millet and soya bean flours. A Higher  $L^*$  value of 74.48 and the hue angle value approaching 90 degrees shows that the shade is lighter and tends to be whiter.

Bulk density is a result of particle density (including occluded air). The optimized flour had a bulk density of  $0.53 \pm 0.01$  g/ml. Regular spray-dried non-fat dry milk is about 0.50–0.60 g/ml and it is reported that the bulk density for legume flours varied from 0.543 g/mL to 0.816 g/mL [41]. The lower the bulk density value, the higher the number of flour particles that can stay together and thus increasing the energy content that could be derivable from such diets [42] (Table 11).

**Table 10:** Proximate composition and per cent daily value of composite flour pre mix

Particulars	Composition (Mean $\pm$ SD)	% Daily value (as per ICMR, 2020) for women
Moisture %	$6.01 \pm 0.08$	--
Crude protein (dry matter basis)	$17.06 \pm 1.2$	37.09
Ether extract (crude fat)(dry matter basis)	$5.21 \pm 0.03$	20.8
Crude fibre (on dry matter basis)	$3.67 \pm 0.18$	12.23
Total ash (dry matter basis)	$2.96 \pm 0.23$	--
Carbohydrate (dry matter basis)	$65.09 \pm 1.4$	--
Calcium (dry matter basis)	$1.13 \pm 0.16$	100
Phosphorus (dry matter basis)	$0.72 \pm 0.08$	--
Energy value	$375.43 \pm 1.77$ Kcal/100g	17.63

\*Figures are the average of three replications

**Table 11:** Physical attributes of the optimized composite flour.

Parameter	Values
Hunter L,a,b	
$L^*$	$74.48 \pm 0.34$
$a^*$	$2.50 \pm 0.07$
$b^*$	$16.77 \pm 0.07$
Hue	$81.51 \pm 0.27$
Bulk density (g/ml)	$0.53 \pm 0.01$
Water Absorption Capacity (%)	$226 \pm 0.03$

Figures are mean  $\pm$  SD of three replications

Water absorption capacity refers to the ability of the flour or starch to hold water against gravity that can comprise bound water, hydrodynamic water, capillary water and physically entrapped water [43]. The observed reading of  $226 \pm 0.03$  % is in accordance with previous studies [44]. A high value indicates that the flour has more hydrophilic components like polysaccharides and protein that interacts more with water. This property indicates its suitability in making porridges.

## Conclusion

Adequate nutritional attainment is equally important for both men and women, but women need additional stress in this regard as nourishing her brings about a healthy generation. The current study was to formulate and optimize composite flour pre-mix for women based on their nutrient requirements ICMR 2020, that an average Indian woman weighing about 55 Kg needs 2130 kilocalories of energy per day, which includes 46 g protein and 25 g visible fat. It was prepared by combining selected cereals, pulses and oilseeds which are nutrient-rich, economical and locally available. The composite mix had high protein and calcium content with considerable other nutrients and could meet about 37% of the daily value of protein, 20% of the daily fat requirement, 12% of the crude fiber, 100% of calcium and 17% of the energy value for Indian women. The phytate content in cereals may hinder the absorption of minerals, though partial roasting may reduce its content of phytic acid [45]. Further studies are warranted in bioavailability and storage studies. The new composite flour mix can be utilized to combat the deep-rooted food insecurity and malnutrition in women, especially in working women.

## Acknowledgements

The author would like to acknowledge the faculty of Dairy Science, Kerala Veterinary and Animal Sciences University, Wayanad for providing the facilities for this work at the Department of Dairy Technology, Verghese Kurien Institute of Dairy and Food Technology (VKIDFT), Thrissur, Kerala as part of the Ph.D. program.

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