# CHAPTER 4

# Formulation of novel value added Baked products: Cookies and Bread

*"Any food that requires enhancing by the use of chemical substances should in no way be considered a food." - John H. Tobe*

## 4.1. Introduction

Baked products like Cookies and bread are considered to be among the most popular, cheap, and easily accessible snack food worldwide (Mohd Basri *et al.,* 2020). Many countries are also using cookies and bread as functional foods for nutrient supplement. For the formulation of such products, optimization process is considered to be a vital step and Response surface methodology (RSM) is proven as potential tool to process optimization. This statistical model has been testified as best model to study interaction and optimization. RSM has been used widely in many studies for the formulation of food products. Mohd Basri et al. (2020) used RSM to formulate oat-based cookies based upon central composite design. Previously studies are carried out regarding value addition of cookies such as with wheat and oat bran (Milićević *et al.,* 2020), rice bran (Sohail *et al.,* 2017).

Since these food products are important part of diet in daily life, therefore ways to improve quality and shelf life is of utmost importance among the bakery products (Chaudhary *et al.,* 1991).

Irradiation technique can enhance the shelf-life of processed food, without altering the nutritional quality of the food product which does not affect the sensory scores (Indiarto and Qonit, 2020). The technology of irradiation in the food domain besides preserving foods, can also sterilize certain food products and maintain food security (Garcia and Copetti, 2019).

Our present interest is to formulate value added baked products viz., cookies and bread followed by their characterization and to understand the role of gamma irradiation technology to improve storage stability of food product. In the following section, we will discuss about the nutrient composition of value added cookies and bread as potential functional food followed by estimation of sensory attributes and shelf life of prepared cookies and breads.

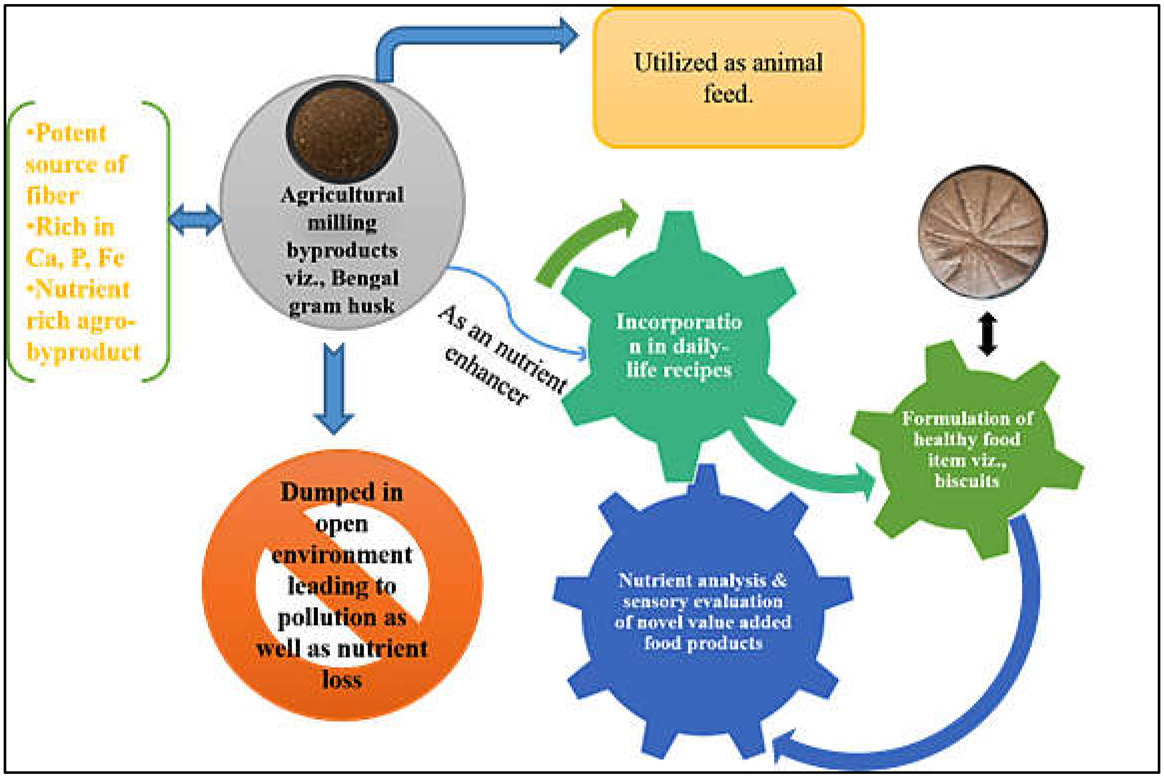


**Figure 4. 1: Utilization of milling by-products to formulate baked products**

### 4.2. Processing of selected milling by-products for multibran cookies formulation

Physico-chemical composition of the raw ingredients, used for the value added product formulation, already had been discussed in Chapter 3. The processed by-product-flours were then subjected to formulation of composite flour according to the experimental design using Response Surface Methodology (RSM). Further development of value added functional bakery products and their characterization had been discussed.

### 4.3. Experimental design to formulate cookies

Based upon the central composite rotatable design (CCRD) of RSM, variables and responses were designed. Literature have been reviewed for optimization of suitable concentration of rice bran flour, wheat bran flour, broken rice flour to develop a desirable product (Sohail *et al.,* 2017, Hassanzadeh-Rostami *et al.,* 2020, Tiwari *et al.,* 2011**).** For the preparation of composite flour, chickpea husk flour and moong bean husk flour were considered as independent variables and the concentration of rice bran flour, wheat bran flour, broken rice flour were maintained at (20±2) %, (40±1) %, (10±2) % respectively. Upper and lower range for chickpea husk flour and moong bean husk flour were nominated between 5-15% and 10-25%, respectively. Selected as responses in the design are taste, texture and overall acceptability. The CCRD was designed (Figure 4.2) into thirteen experiments with four factorial points along with five replicates and four axial points.

Responses were determined utilizing regression analysis:

$$\boldsymbol{Y = \beta_0 + \sum_{i=1}^{n} \beta_i X_i + \sum_{i=1}^{n} \beta_{ii} X_i^2 + \sum_{i \neq j=1}^{n} \beta_{ij} X_i X_{ij}} \quad \text{............................. Eq. 4.1}$$

Where $\beta_0$= value of response at the centre points (0,0), $X_i$, Xij= variables of design, $\beta_i$, $\beta_{ii}$, $\beta_{ij}$= regression coefficients, n= number of variables.

### 4.3.1. Data analysis

For the optimization of composite flour formulation, State-Ease Design expert software (Ver. 13.0) was used. Two function variables were considered to plot the response surfaces and 3D- contour graphs. Regarding the accuracy of the designed model coefficient of determination was estimated (significant level of $p \leq 0.05$).

## 4.4. Formulation of value added cookies

The basic ingredients were the by-product (chickpea husk, moong bean husk, rice bran, wheat bran, broken rice) flours, wheat flour, jaggery (traditional non-centrifugal cane sugar), vegetable oil, salt, and baking powder (Figure 4.2) to formulate Multibran cookies. The product was formulated by partial substitution (40%) of the wheat flour with the prepared composite flour. The dry ingredients were kneaded with creamy mixture of sugar and vegetable oil (Okaka, 1997) for 6-7 min. using kneader. The formed dough was shaped out by a cookies cutter (3.6 X 3.6″). After the baking, the cookies were stored in polyethylene bags at room temperature for further uses. Same procedure was followed to formulate mushroom cookies and 'Control' wheat flour cookies to get a comparative study with novel multibran cookies. Mushroom cookies were prepared by using mushroom powder and all-purpose wheat flour in 9:1 ratio.

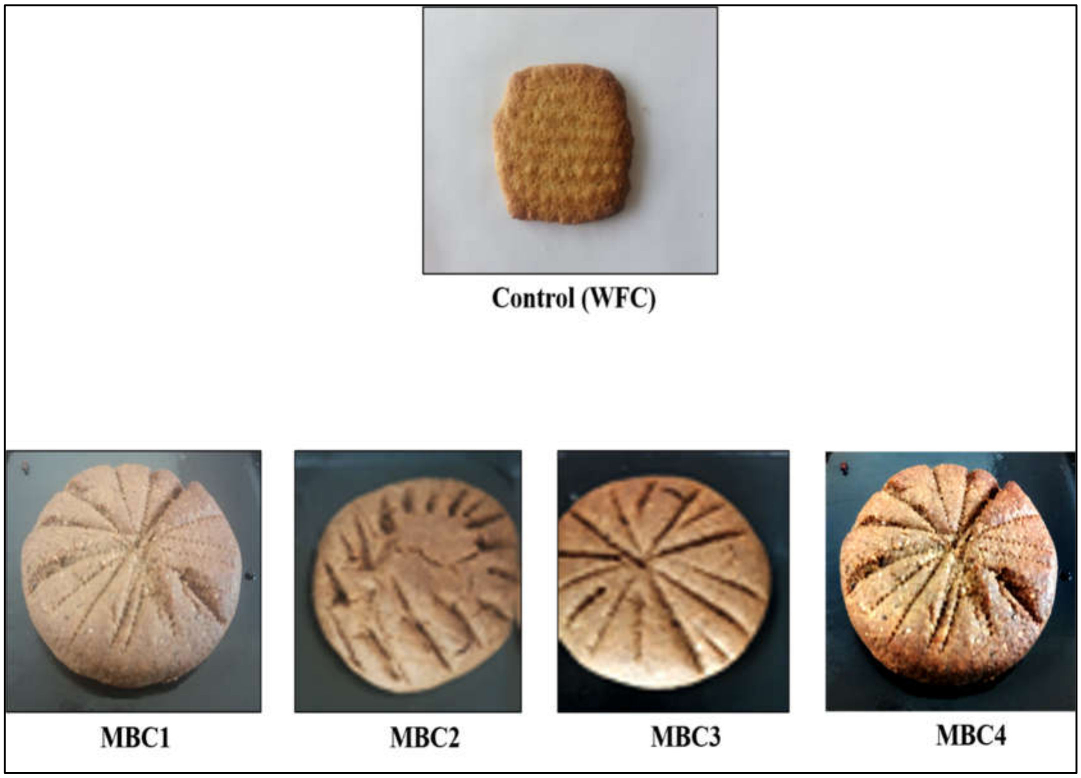


**Figure 4. 2: Formulated nutrient rich variants of Multi-bran Cookies (MBC1, MBC2, MBC3, MBC4) and wheat flour cookies (WFC-control)**

**4.5. Formulation of nutrient rich baked product- Bread**

500 g of whole wheat flour along with 10 g of each of chickpea husk flour, moong bean husk flour, and rice bran flour. 35 g sugar and 10 g salt were mixed. Next, 10 g activated yeast, 250 mL of luke warm water and 70 ml oil were added followed by thorough mixing (5~8 min) to form the dough (Figure 4.2). Upon completion of the dough kneading, it was proofed for 2hrs and baked at 250°C for 15 min in oven. Baked bread was packed in a polyethylene bag to retain the moisture and soften the bread crumb. Then it was left to cool down for further analysis. Same method was followed to prepare mushroom bread, where all purpose wheat flour and mushroom flour ratio was 9:1.

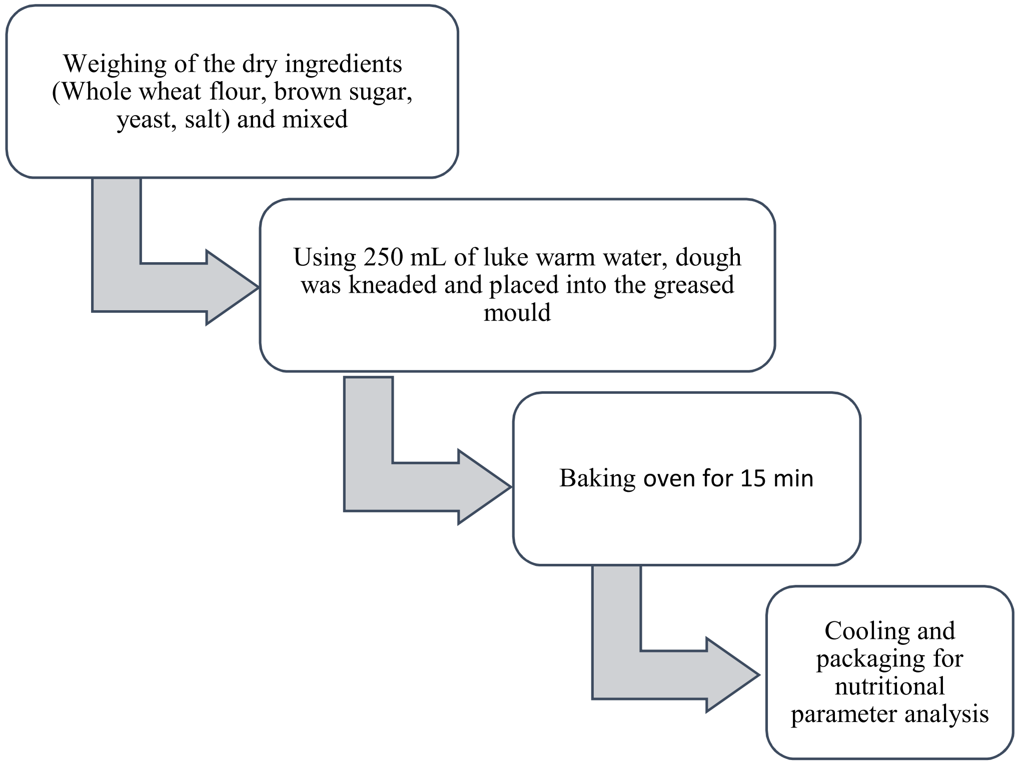


**Figure 4. 3: Preparation of different types of value added breads**

| Ingredients (g) | BBr0 (g) (Control) | BBr1 (g) | BBr2 (g) | BBr3 (g) | BBr4 (g) |
|---|---|---|---|---|---|
| Rice bran flour | 0 (0%) | 10 (1.87%) | 20 (3.57%) | 30 (5.04%) | 40 (6.45%) |
| Chickpea husk flour | 0 (0%) | 10 (1.87%) | 20 (3.57%) | 30 (5.04%) | 40 (6.45%) |
| Moong bean husk | 0 (0%) | 10 (1.87%) | 20 (3.57%) | 30 (5.04%) | 40 (6.45%) |
| Wheat flour | 500 (100%) | 500 (94.34%) | 500 (89.29%) | 500 (84.75%) | 500 (80.65%) |

**Table 4. 1: Ingredient composition to formulate value added Bran Bread (BBr).**

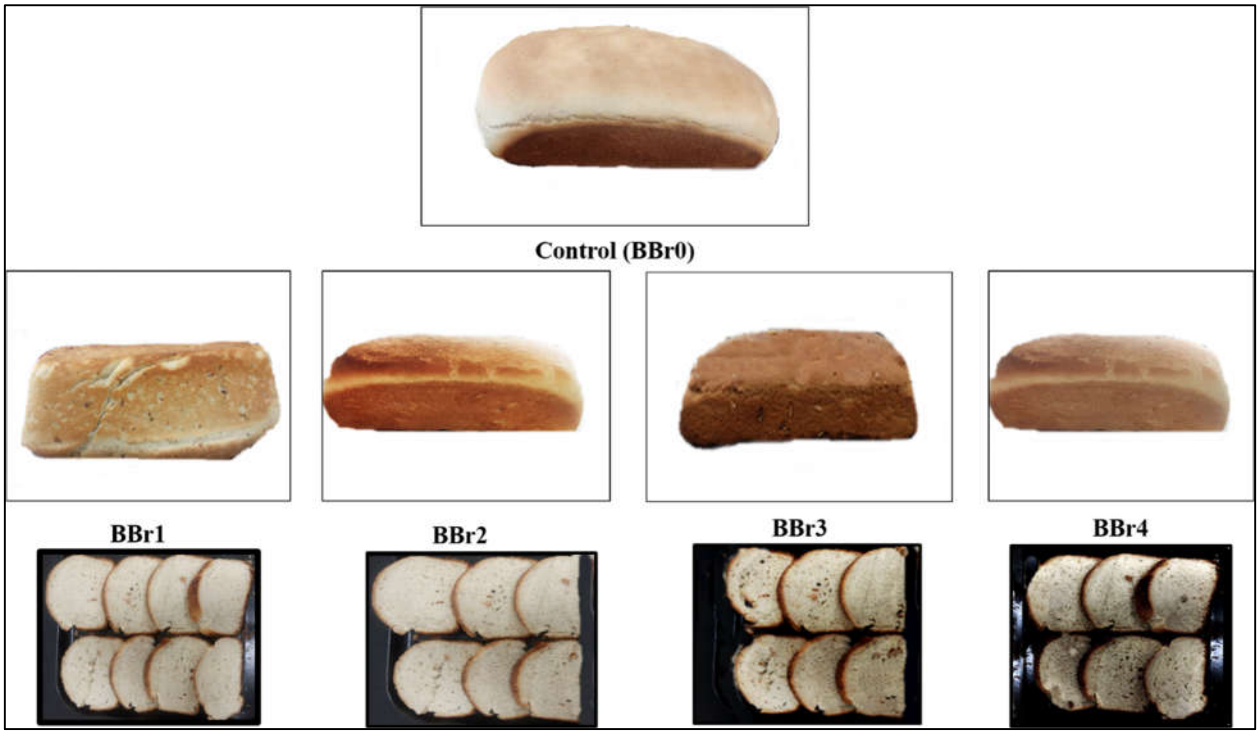


**Figure 4. 4: Formulated nutrient rich variants of Bran Bread (BBr0, BBr1, BBr2, BBr3, BBr4) using different percentage composition of milling by-products.**

After the formulation of the baked products, cookies and breads, they were characterized based upon nutrient composition, sensory parameters, and shelf life study. Further the products were exposed to gamma sources to understand the effect on the storage stability of the formulated products.

### 4.6. Irradiation of the formulated bakery products

The products were exposed to $^{137}Cs$ and $^{60}Co$ gamma sources to improve the storage stability of the products. Formulated breads and cookies were irradiated after 3 days of the manufacturing date. The samples used to study the shelf life along with their densities are stated below.

| S.N. | Sample | Density (g/cm$^3$) |
|---|---|---|
| 1 | Bran Bread | 0.515 |
| 2 | Mushroom Bread | 0.344 |
| 3 | Multibran cookies | 0.763 |
| 4 | Mushroom cookies | 0.708 |
| 5 | Wheat Flour cookies | 0.708 |

**Table 4. 2: Density of the formulated plant source based value added products**

The evaluation of the shelf life of irradiated bread, cookies, and control was made from the observation of the visual aspects. The samples were observed continuously each passing day till mold started growing on the bread samples and that was considered as initiation of the spoilage of bread.

**4.7. Results and discussions**

After the development of the functional products, we here analysed the characterization of the products followed by the study of shelf life and sensory parameters.

**4.7.1. Analysis of design and characterization of formulated cookies**

Evaluation of the regression equations of response was done to check the model fitting of RSM. Regression equation was studied to understand the interactive effect of the variables. The suitability of linearity and quadratic combination of variables was described by examining the data with reference (Table 4.3).

| **Source** | **Sum of Squares** | **Df** | **Mean Square** | **F-value** | **p-value** |
|---|---|---|---|---|---|
| **Texture** | | | | | |
| **Model (RSM)** | 0.4480 | 2 | 0.2240 | 14.03 | 0.0013 (significant) |
| A-Chickpea husk flour | 0.3747 | 1 | 0.3747 | 23.46 | 0.0007 |
| B-Moong bean husk flour | 0.0733 | 1 | 0.0733 | 4.59 | 0.0578 |

| **Source** | **Sum of Squares** | **Df** | **Mean Square** |
|---|---|---|---|
| **Residual** | 0.1597 | 10 | 0.0160 |
| Lack of Fit | 0.1597 | 6 | 0.0266 |
| Pure Error | 0.0000 | 4 | 0.0000 |
| **Cor Total** | 0.6077 | 12 | ---- |

**Table 4. 3: Analysis of variance (ANOVA) result for the optimization of variable (Texture) to formulate multibran cookies**

| Response | | | | | |
|---|---|---|---|---|---|
| **Source** | **Sum of Squares** | **Df** | **Mean Square** | **F-value** | **p-value** |
| **Model (RSM)** | 0.8511 | 2 | 0.4255 | 17.75 | 0.0005 (Significant) |
| A-Chickpea husk flour | 0.7243 | 1 | 0.7243 | 30.21 | 0.0003 |
| B-Moong bean husk flour | 0.1268 | 1 | 0.1268 | 5.29 | 0.0443 |

| **Source** | **Sum of Squares** | **Df** | **Mean Square** |
|---|---|---|---|
| **Residual** | 0.2397 | 10 | 0.0240 |
| Lack of Fit | 0.2397 | 6 | 0.0400 |
| Pure Error | 0.0000 | 4 | 0.0000 |
| **Cor Total** | 1.09 | 12 | ----- |

**Table 4. 4: Analysis of variance (ANOVA) result for the optimization of responses to formulate multibran cookies**

| Overall acceptability | | | | | |
|---|---|---|---|---|---|
| **Source** | **Sum of Squares** | **Df** | **Mean Square** | **F-value** | **p-value** |
| **Model (RSM)** | 0.6271 | 2 | 0.3135 | 10.32 | 0.0037 (Significant) |
| A-Chickpea husk flour | 0.5334 | 1 | 0.5334 | 17.56 | 0.0019 |
| B-Moong bean husk flour | 0.0937 | 1 | 0.0937 | 3.08 | 0.1096 |
| **Source** | | **Sum of Squares** | | **Df** | **Mean Square** |
| **Residual** | | 0.3037 | | 10 | 0.0304 |
| Lack of Fit | | 0.3037 | | 6 | 0.0506 |
| Lack of Fit | | 0.3037 | | 6 | 0.0506 |
| Pure Error | | 0.0000 | | 4 | 0.0000 |
| **Cor Total** | | 0.9308 | | 12 | ---- |

**Table 4. 5: Analysis of variance (ANOVA) result for the optimization of variable (Overall acceptability) to formulate multibran cookies**

Based upon the three dimensional graphs of the responses it is observed that Texture quality was higher when level of variation moves towards the axial position of high moong bean husk proportion. Comparatively lesser substitution of chickpea husk was seemed to be associated with higher texture quality (Figure 4.6). During previous study evaluation by Niño-Medino et al. (2019), change in sensory parameters of composite flour (utilized for the formulation of the cookies) was noticed due to variation in different legume substitution. Same pattern was also observed in case of another response, taste parameter (Figure 4.8**)**. In case of overall acceptability, the three dimensional graph showed higher score toward the axial portion of moong bean husk and chickpea husk substitution (Figure 4.9). Saddle points were pointed out to get the optimized value of the response variables within the given range of formulated value-added products.

| Std | Run | Space Type | Factor 1 A:Chickpea husk... % | Factor 2 B:Moong bean ... % | Response 1 Texture | Response 2 Taste | Response 3 Overall acceptability |
|---|---|---|---|---|---|---|---|
| 1 | 3 | Factorial | 5 | 10 | 8 | 8.2 | 8.3 |
| 2 | 2 | Factorial | 15 | 10 | 7.6 | 7.5 | 7.7 |
| 3 | 4 | Factorial | 5 | 25 | 8 | 8.5 | 8.6 |
| 4 | 10 | Factorial | 15 | 25 | 7.8 | 7.5 | 7.7 |
| 5 | 11 | Axial | 2.92893 | 17.5 | 8 | 8 | 8 |
| 6 | 8 | Axial | 17.0711 | 17.5 | 7.2 | 7.5 | 7.6 |
| 7 | 7 | Axial | 10 | 6.8934 | 7.5 | 7.5 | 7.6 |
| 8 | 5 | Axial | 10 | 28.1066 | 7.9 | 8 | 8 |
| 9 | 12 | Center | 10 | 17.5 | 7.8 | 7.9 | 8 |
| 10 | 1 | Center | 10 | 17.5 | 7.8 | 7.9 | 8 |
| 11 | 13 | Center | 10 | 17.5 | 7.8 | 7.9 | 8 |
| 12 | 9 | Center | 10 | 17.5 | 7.8 | 7.9 | 8 |
| 13 | 6 | Center | 10 | 17.5 | 7.8 | 7.9 | 8 |

**Figure 4. 5: Central composite design arrangement to optimize the composite flour preparation of value added nutrient rich Multibran cookies**

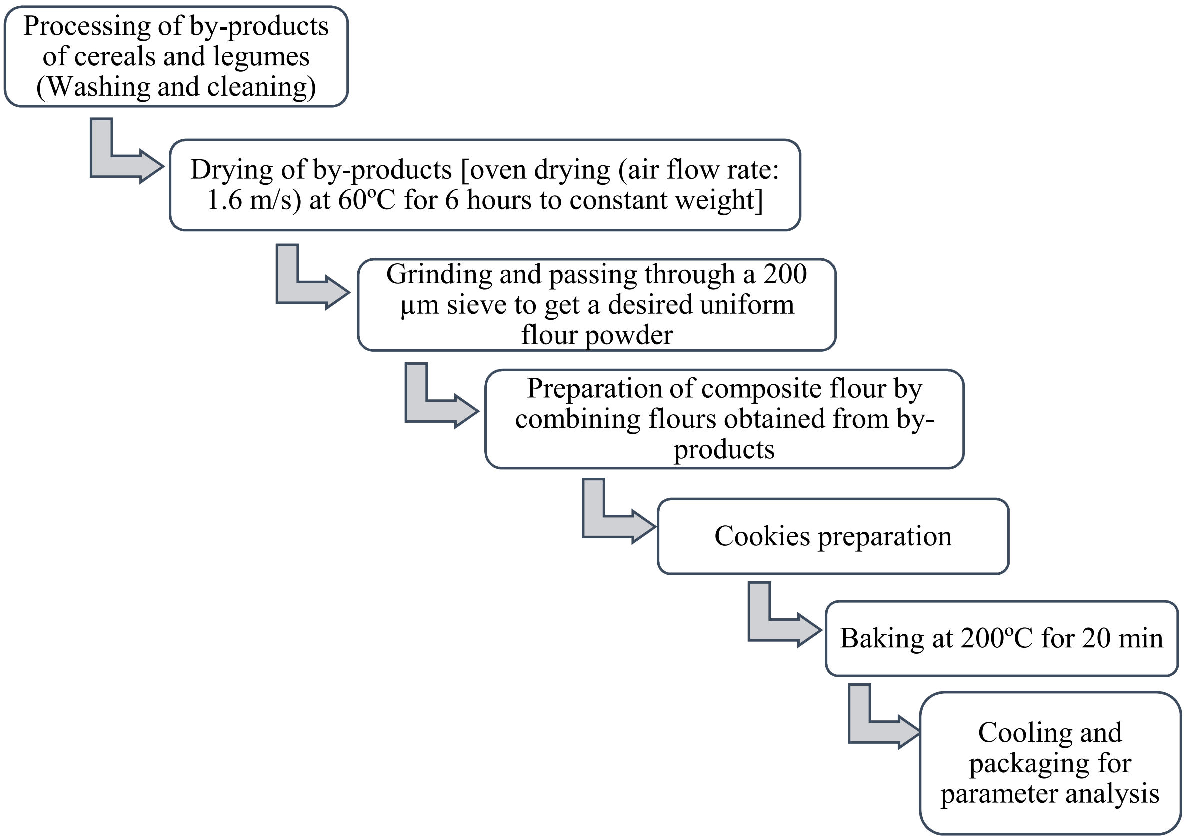


**Figure 4. 6: Preparation of cookies substituted with cereal-legume husk flour**

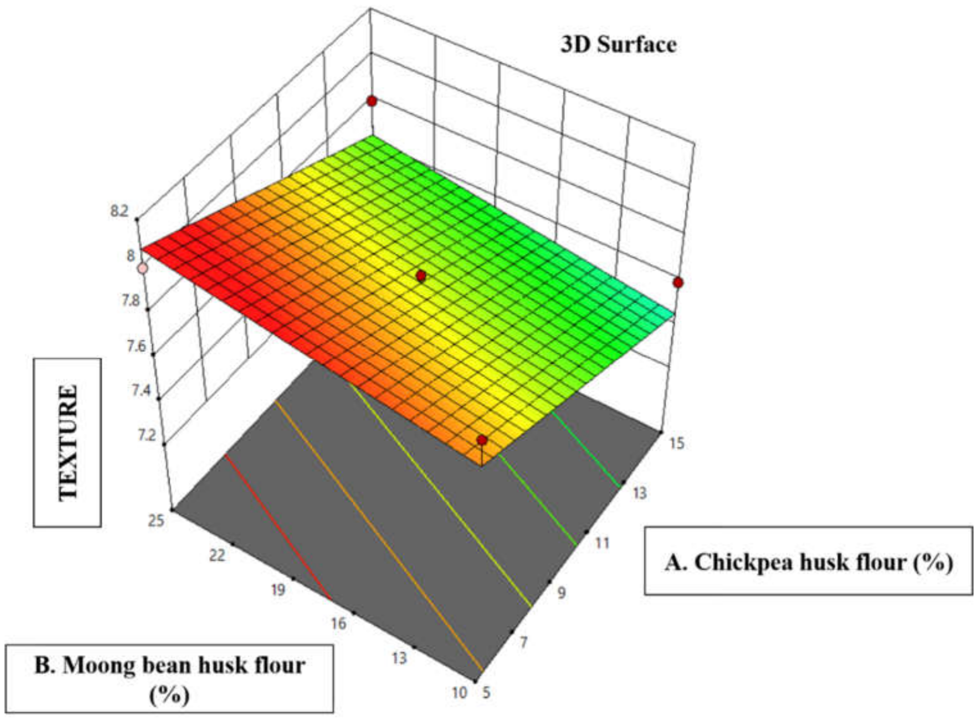


**Figure 4. 7: Observation obtained from the optimized design: effect of variables on Texture of value added nutrient rich Multibran cookies**

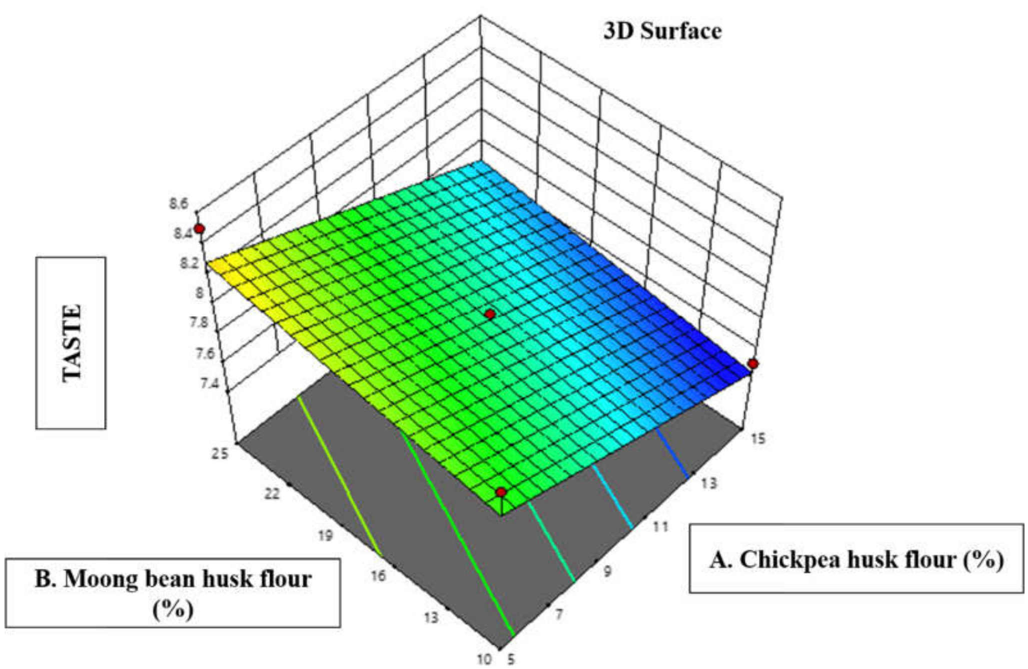


**Figure 4. 8: Observation obtained from the optimized design: effect of variables on Taste of value added nutrient rich Multibran cookies**

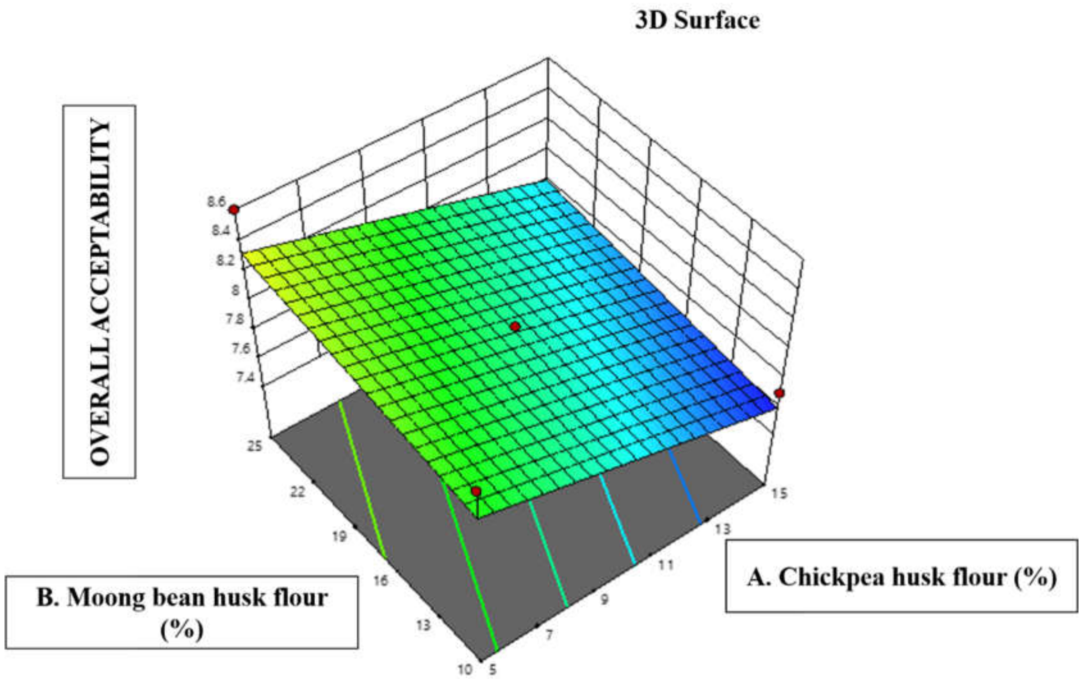


**Figure 4. 9: Observation obtained from the optimized design: effect of variables on Overall acceptability of value added nutrient rich Multibran cookies**

#### 4.7.1.1.Optimization and justification of response variables

Based on the response values to definite level (obtained using State-Ease Design expert software Ver. 13.0), model design conveyed the data at various level of substitutions of variables. These obtained data were further used for actual experimental responses. Same pattern was also disclosed in a study (Mohd Basri *et al.* 2020). According to the data provided by CCRD design, the optimized value of independent variables for composite flour obtained to formulate cookies were 10% of chickpea husk flour and 17.5% moong bean husk flour.

#### 4.7.1.2. Detailed study of nutritional compositions of MBC Cookies

##### 4.7.1.2.1. Proximate compositions

Addition of protein and fibre rich legume husk in the formulation, showed enhanced protein level almost by 50% accompanied by a significant increase ($p \leq 0.05$) in fibre in comparison with the control i.e. wheat flour cookies (WFC) **(**Table 4.6). Crude protein content (%) in MBC and WFC was estimated (18.01±0.01) % and (7.78±0.06) % respectively. Moisture present in MBC was around 5%. However, the moisture content of MBC was higher than that of WFC. Higher moisture content in comparison with control might attributed to the fact of degradation of carbohydrates. Besides, lower carbohydrate level also contributes to the increase absorption of oil to form an appropriate cookies dough (Singh Sibian and Singh Riar, 2020). Wheat flour cookies contained higher moisture (4.05±0.02%) than that of mushroom cookies (2.7±0.33%) and multibran cookies (5.10±0.07%). Crude fat (%) was found lesser in MBC [(4.54±0.04) %] than WFC [(17.98±0.19) %]. Mushroom cookies contained higher crude fat (12.01±0.01%) than multibran cookies. The Proximate composition of novel MBC cookies was found to be enhanced. Consumption of MBC might attribute to improved health management than the wheat flour cookies.

| Proximate composition | Multi- bran cookies (MBC) | Wheat flour cookies (WFC) | Mushroom cookies |
|---|---|---|---|
| **Moisture* (%)** | 5.10±0.07$^{a}$ | 4.05±0.02$^{b}$ | 2.7±0.33$^{c}$ |
| **Crude protein (%)** | 18.01±0.01$^{a}$ | 7.78±0.06$^{c}$ | 8.9±1.11$^{b}$ |
| **Crude fat (%)** | 4.54±0.04$^{c}$ | 17.98±0.19$^{a}$ | 12.01±0.01$^{b}$ |
| **Crude fibre (%)** | 5.01±0.06$^{a}$ | 2.01±0.02$^{b}$ | 1.5±0.05$^{c}$ |
| **Ash (%)** | 6.56±0.10$^{a}$ | 1.10±0.02$^{c}$ | 1.70±0.09$^{b}$ |
| **Total carbohydrate (%)** | 59.82±2.01$^{c}$ | 70.47±0.01$^{a}$ | 69.19±0.01$^{b}$ |

**Table 4.6: Proximate composition of the formulated value added products- Multibran cookies, Mushroom cookies and Wheat flour cookies [Significantly different, $p < 0.05$ (Statistical analysis has been done row wise)].**

#### 4.7.1.2.2. Minerals

Ash content (%) was significantly higher ($p \leq 0.05$) in MBC (6.56±0.10) % than WFC (1.10±0.02) %. Iron (16.89±0.08 mg/100g), calcium (115.06±4.03 mg/100g), and phosphorus (195.88±0.20 mg/100g) levels were found considerably higher than control (WFC) (Table 4.7). Enhanced nutrient composition due to legume incorporation was acknowledged by Niño-Medino et al. (2019). Presence of jaggery, moong bean husk, and rice bran in the formulation can contribute in improved iron level. According to a study by

Sohail et al. (2017), rice bran contains significant amount of iron**.** Micronutrients are essential for the functioning of living. In WFC, Iron content was 1.05±0.06 mg/100g, calcium level was 6.07±0.01 mg/100g, and phosphorus found was 21.22±0.01 mg/100g, which are lesser than minerals present in MBC. Hence, micronutrient-rich MBC can be an addition to a nutritious snack diet to enhance health status. Although there are micronutrient rich products available in market, they also provide high calorie which increases risk of overweight or metabolic disorders upon consumption.

| **Nutrient composition** | **Multi- bran cookies (MBC)** | **Wheat flour cookies (WFC)** |
|---|---|---|
| **Non-Reducing sugar (g/100 g)** | $0.91 \pm 0.01^{b}$ | $1.94 \pm 0.01^{a}$ |
| **Reducing sugar (g/100 g)** | $2.17 \pm 0.18^{b}$ | $2.95 \pm 0.02^{a}$ |
| **Total soluble sugar (g/100 g)** | $3.08 \pm 0.05^{b}$ | $4.89 \pm 0.01^{a}$ |
| **Starch (g/100 g)** | $26.08 \pm 0.04^{b}$ | 27.01 ± 0.01 |
| **Iron (mg/100g)** | $16.89 \pm 0.08^{a}$ | $1.05 \pm 0.06^{b}$ |
| **Calcium (mg/100g)** | $115.06 \pm 4.03^{a}$ | $6.07 \pm 0.01^{b}$ |
| **Phosphorus (mg/100g)** | $195.88 \pm 0.20^{a}$ | $21.22 \pm 0.01^{b}$ |
| **Trypsin inhibitor activity (TIU/mg)** | $1.71 \pm 2.01^{a}$ | $1.02 \pm 1.06^{b}$ |
| **Phytic acid (mg/100 g)** | $293.01 \pm 1.32^{a}$ | $140.02 \pm 1.12^{b}$ |
| **Total phenolic content (mg GAE/ g)** | $3.11 \pm 0.05^{a}$ | $1.59 \pm 0.22^{b}$ |
| **DPPH Radical Scavenging Activity (%)** | $45.99 \pm 0.05^{a}$ | $12.03 \pm 0.25^{b}$ |
| **In vitro digestibility** of **Starch (mg maltose released/ g of product starch)** | $30.51 \pm 0.07^{b}$ | $32.99 \pm 0.05^{a}$ |
| **In vitro digestibility** of **Protein (%)** | $69.89 \pm 0.15^{a}$ | $52.01 \pm 0.01^{b}$ |
| **Soluble dietary fibre(g/ 100g)** | $6.85 \pm 0.12^{a}$ | $0.98 \pm 0.01^{b}$ |
| **Insoluble dietary fibre (g/ 100g)** | $3.53 \pm 0.11^{a}$ | $1.04 \pm 0.02^{b}$ |
| **Total dietary fibre (g/ 100g)** | $10.38 \pm 0.06^{a}$ | $2.02 \pm 0.05^{b}$ |

**Table 4. 7: Analysed nutrient composition of formulated product- Cookies per 100g- Multi-bran cookies, mushroom cookies and wheat flour cookies [Significantly different, $p < 0.05$ (Statistical analysis has been done row wise)].**

#### 4.7.1.2.3. Antinutrient

Trypsin inhibitor activity was found 1.71 ± 2.01 TIU/mg in MBC along with 293.01 ± 1.32 mg/100g of phytic acid content (Table 4.7). MBC showed negligible antinutrient content in comparison to source. This might occur due to various processing techniques used viz., washing, soaking, blanching during preparation of the product. Trypsin inhibitor activity decreased significantly **(**Shi *et al.,* 2018**).**

**4.7.1.2.4. Phenolic compounds and antioxidant properties**

Phenolic content and DPPH RSA for the MBC were 3.11 ± 0.05 mg GAE/g and (45.99 ± 0.05) % respectively. Whereas, the control type (WFC) showed 1.59 ± 0.22 mg GAE/g Total Phenolic content with minimal DPPH Radical Scavenging Activity [(12.03 ± 0.25) %] (Table 4.7). Improved phenolic content and antioxidant activity was reported in chickpea husk based bread (Niño-Medina *et al.,* 2019). Presence of wheat bran in the product might contribute to the rich antioxidant property **(**Higuchi 2014**).** Blanching, heating in traditional oven or microwave oven affect the antioxidant property of prepared products (Minatel *et al.,* 2017).

**4.7.1.2.5. In vitro protein and starch digestibility**

Estimated in vitro starch digestibility of MBC was 30.51±0.07 mg maltose released/g of product starch and that of WFC was 32.99±0.05 mg maltose released/g of product starch. Higher in vitro protein digestibility (%) of MBC [(69.89±0.15) %] (Table 4.7) was recorded. Gluten containing wheat flour cookies (Control) showed lesser in vitro protein digestibility which was (52.01±0.01) %. Rich in vitro digestibility of starch and protein was also disclosed (Singh Sibian and Singh Riar , 2020) due to addition of germinated legume grains. Market cookies are generally 100% wheat flour based products, whereas MBC is almost gluten-free product. Therefore, novel MBC cookies can be a nutritive baked snack to boost digestibility.

**4.7.1.2.6. Dietary fibre**

Incorporation of composite flour in cookies resulted in increased soluble dietary fibre (g/ 100g) of MBC (6.85±0.12) in comparison to WFC (0.98±0.01). Insoluble dietary fibre (g/ 100g) level was also elevated in MBC (3.53±0.11). Total dietary fibre (g/ 100g) of the formulated MBC and WFC was 10.38±0.06 g/ 100g and 2.02±0.05 g/ 100g respectively (Table 4.7). Enhanced dietary fibre was also noted after use of chickpea husk fibre in food production (Niño-Medino *et al.,* 2019). Formulated MBC might contribute to fibre requirement resulting in improved metabolic health conditions.

#### 4.7.1.2.7. Sensory evaluation of cookies

The overall acceptability was more in case of WFC (8.26) than MBC (8.134), although MBC showed satisfactory acceptability (Figure 4.10**)** in sensory profiling among panellists. Niño-Medino et al. (2019) and Bora and Kulshrestha, K. (2015) in their study concluded that the chickpea husk and moong bean husk based product formulations exhibited satisfactory acceptability among consumers .

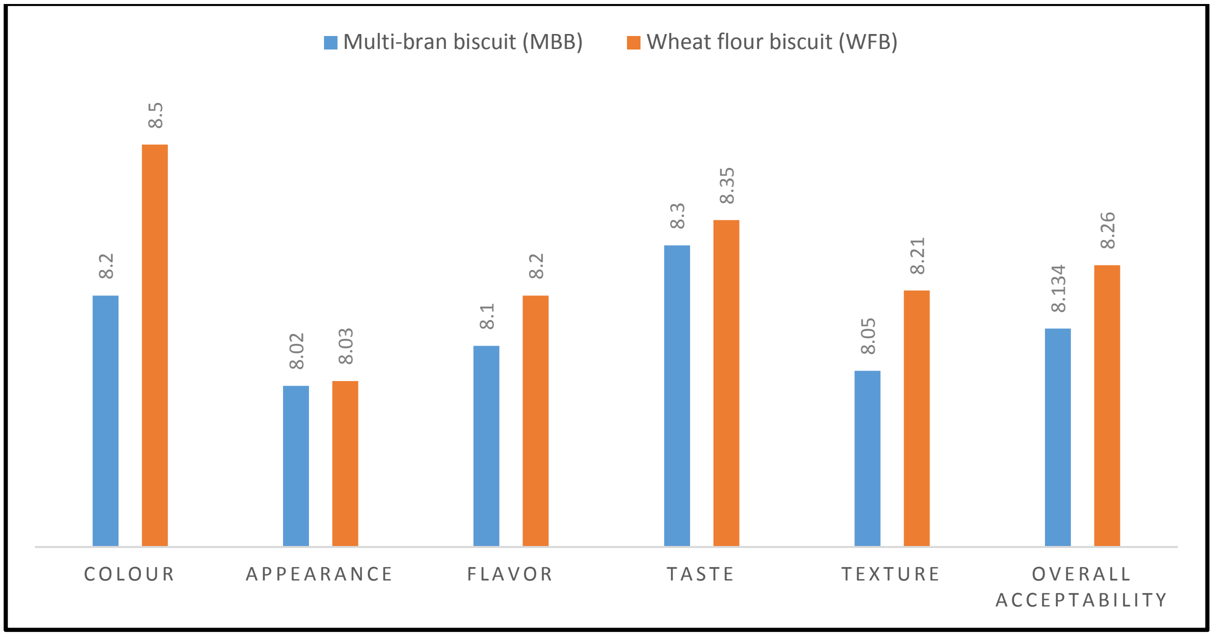


**Figure 4. 10: Sensory parameters of Freshly prepared Multi-Bran Cookies and Wheat Flour Cookies**

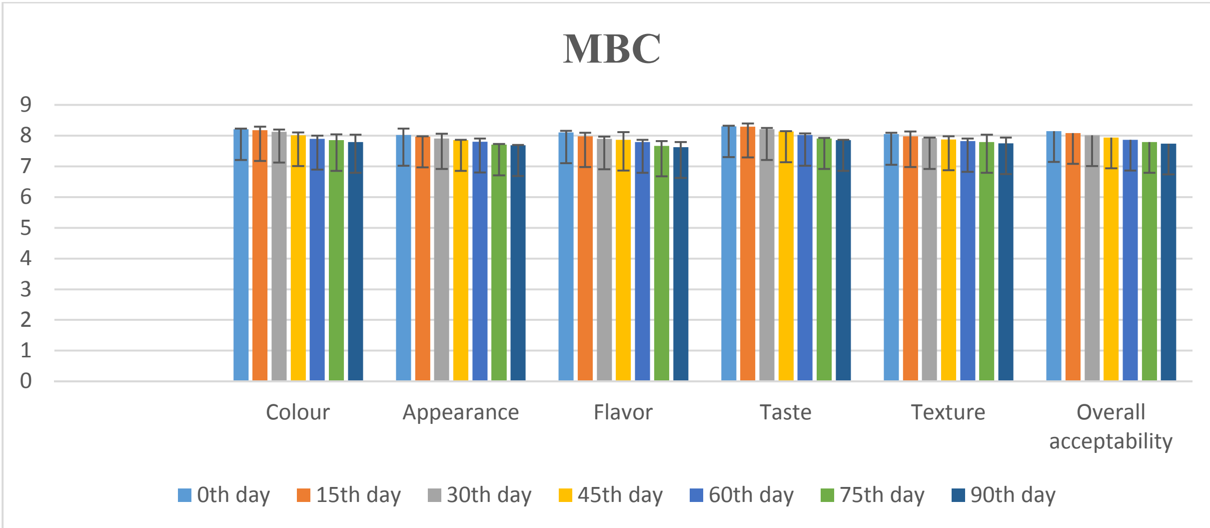


**Figure 4. 11: The effect of storage on sensory quality characteristics of Products-Multibran cookies**

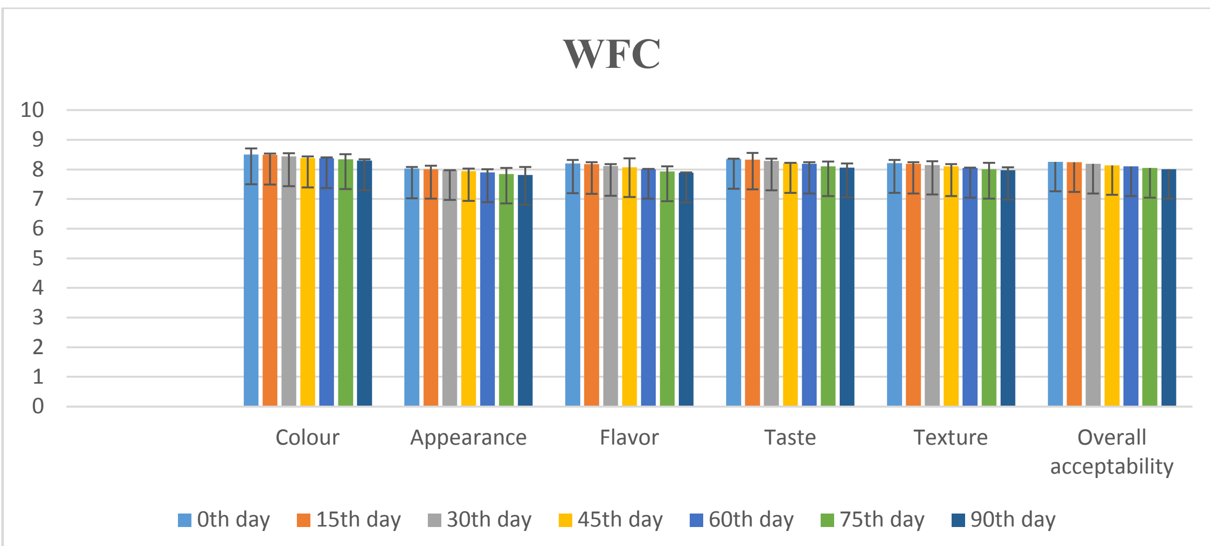


**Figure 4. 12: The effect of storage on sensory quality characteristics of Products-wheat flour cookies**

### 4.7.1.3.Shelf life of the prepared food products

According to Figure 4.11 and 4.12, the sensory score of the products retained till $90^{th}$ day. 2 meq peroxide/1000g and 12 meq peroxide/1000g peroxide values were recorded on $1^{st}$ and $90^{th}$ day of the storage duration (Table 4.5). Free fatty acid value was found to be 0.3 to 0.8 mg KOH/100g in these products during the storage. Analysed data indicated higher shelf life of the products.

#### 4.7.1.4. Microbiological safety of food

In MBC and WFC, Total Plate Counts (Log CFU/g) were found to be 2.11 ± 0.01 and 2.07 ± 0.12 respectively (Table 4.9). After wards during the storage of the product, total Plate Count (Log CFU/g) increased to 3. No yeast and mold count was detected in fresh preparation. On 75$^{th}$ day the yeast count (Log CFU/g) increased to 2.07 ± 0.05 and 2.11 ± 0.04 in MBC and WFC respectively.

| Day intervals | 0$^{th}$day | 15$^{th}$day | 30$^{th}$day | 45$^{th}$day | 60$^{th}$day | 75$^{th}$day | 90$^{th}$day |
|---|---|---|---|---|---|---|---|
| **Multibran cookies** | | | | | | | |
| Peroxide value (meq peroxide/1000g | 2.24±0.01$^{e}$ | 2.65±0.08$^{b}$ | 4.98±0.04$^{c}$ | 6.78±0.11$^{a}$ | 8.91±0.01$^{e}$ | 11.05±0.04$^{c}$ | 12.98±0.03$^{d}$ |
| Free fatty acid (mg KOH/100g) | 0.52±0.08$^{b}$ | 0.61±0.10$^{a}$ | 0.69±0.12$^{a}$ | 0.75±0.11$^{a}$ | 0.79±0.16$^{c}$ | 0.82±0.08$^{b}$ | 0.90±0.02$^{d}$ |
| **Wheat flour cookies** | | | | | | | |
| Peroxide value (meq peroxide/1000g | 2.01±0.01$^{e}$ | 3.05±0.08$^{b}$ | 4.89±0.04$^{c}$ | 6.94±0.11$^{a}$ | 8.89±0.01$^{e}$ | 10.87±0.04$^{c}$ | 12.12±0.03$^{d}$ |
| Free fatty acid (mg KOH/100g) | 0.31±0.08$^{b}$ | 0.42±0.10$^{a}$ | 0.55±0.12$^{a}$ | 0.61±0.11$^{a}$ | 0.73±0.16$^{c}$ | 0.81±0.08$^{b}$ | 0.85±0.02$^{d}$ |

**Table 4. 8: Shelf life determination of novel products. [Significantly different, $p < 0.05$ (Statistical analysis has been done row wise)]**

MBC exhibited higher amount of nutrients, better-quality with satisfactory sensory acceptance. A notable change in nutrient composition, antioxidant activity, and in vitro digestibility of MBC explored probable utilization of milling by-products, especially the by-product of chickpea and moong bean as alternative food source.

| No. of days | MBC | WFC |
|---|---|---|
| **Total Plate Count** | | |
| $0^{th}$ day | 2.11±0.01 | 2.07±0.12 |
| **$15^{th}$ day** | 2.19±0.05 | 2.13±0.05 |
| **$30^{th}$ day** | 2.38±0.12 | 2.25±0.02 |
| **$45^{th}$ day** | 2.52±0.01 | 2.30±0.22 |
| **$60^{th}$ day** | 2.66±0.09 | 2.35±0.19 |
| **$75^{th}$ day** | 2.81±0.21 | 2.42±0.20 |
| **$90^{th}$ day** | 3.07±0.08 | 2.49±0.04 |
| **Yeast and Mold Count** | | |
| **$0^{th}$ day** | -ve | -ve |
| **$15^{th}$ day** | -ve | -ve |
| **$30^{th}$ day** | -ve | -ve |
| **$45^{th}$ day** | -ve | -ve |
| **$60^{th}$ day** | -ve | -ve |
| **$75^{th}$ day** | 2.07±0.05 | 2.11±0.04 |
| **$90^{th}$ day** | 2.15±0.14 | 2.19±0.07 |

**Table 4. 9: Microbial load (Log CFU/g) analysis of freshly formulated product [–ve: not detected].**

### 4.7.2. Characterization of value added breads

Proximate composition of the formulated products has been shown in the Table 4.10. Mushroom bread showed highest moisture that is 27.89±0.01%, whereas that of bran bread was 22.91±1.02%. Mushroom bread (9±0.08%) showed higher fat content than bran bread (2.55±0.01%). Bran bread (12.20±1.03%) contains more crude protein than mushroom bread. Crude fibre was higher in bran products i.e. 5.01±0.06% in multibran cookies and 3.1±0.06% in bran bread. Mushroom bread contained the lowest amount of carbohydrate which is 50.7±2.01%. Enhanced proximate composition due to incorporation of mushroom in bakery products was also observed by Ng et al. (2017) along with improved postprandial glycaemic response. Milling by-products in bakery product development also revealed improved fibre and protein content of the products (Niño-Medina *et al.,* 2019). Yeast fermentation in bread formulation has also showed significant rise in nutrient composition of bread (Koistinen *et al.,* 2018). In a study, Bredariol *et al.* (2020) revealed that due to different baking temperatures nutrient contents especially macronutrients vary.

| Product | Moisture (%) | Ash (%) | Crude fat (%) | Crude protein (%) | Crude fibre (%) | Total carbohydrate (%) |
|---|---|---|---|---|---|---|
| Mushroom bread | 27.89±0.01[a] | 1.05±0.15[b] | 9±0.08[a] | 11.05±1.47[b] | 0.31±0.05[b] | 50.7±2.01[b] |
| Bran bread | 22.91±1.02[b] | 2.48±0.22[a] | 2.55±0.01[b] | 12.20±1.03[a] | 3.1±0.06[a] | 56.76±1.05[a] |

**Table 4.10: Proximate composition of formulated breads [Significantly different, $p < 0.05$ (Statistical analysis has been done column wise)]**

This current study reveals a novel significant research area of nutrient dense product formulation by using processed cereal-legume by-products. Popularization of these products (discussed in Chapter 3) also had been done in collaboration with KrishiVigyan Kendra, Mahendergarh.

#### 4.7.3. Mass attenuation coefficient of formulated products

The mass attenuation coefficient of Bran Bread, Mushroom Bread, Multibran Cookies, Mushroom cookies and Wheat-flour cookies were measured for two different energies of Photons (gamma radiations) and that has been tabulated as follows in Table 4.11. It has been noted that the Mass Attenuation Coefficient gets lower considerably with increasing photon energy. Similar result has been observed in the study in which Mass Attenuation Coefficient of pomegranate peel, lemon peel, pumpkin peel, mandarin peel, grape peel, pineapple peel, orange peel, and grape stalk were measured at different photon energies (Chaudhary *et al.,* 1991). For bread samples, greater shift in Mass Attenuation Coefficient with increasing energy of incident photon is observed in case of Mushroom Bread as compared to the Bran Bread. For Cookies, the shift in Mass Attenuation Coefficient with increasing energy of incident photon is highest in case of Wheat-flour cookies and then Mushroom cookies and least in Bran Bread. The two bakery products (Bread and cookies) differ in structure due to the different baking conditions results into products of different densities. By comparing the Attenuation Coefficient of Bran Bread and Multibran cookies; Mushroom cookies having greater density hence larger value of attenuation coefficient as compared to the Mushroom Bread. Similarly, multibran cookies has greater value of attenuation coefficient as compared to Bran Bread which is in order with increasing value of attenuation coefficient with the increase in density of the sample. Decrease in the value of mass attenuation coefficient with increase in photon energy is attributed to higher photon interaction probability at lower energy and photon interaction probability decreases as the energy of photon increases (Limkitjaroenporn *et al.,* 2012).

| | Energy of Photon 662 KeV | | Energy of Photon 1733 KeV | |
|---|---|---|---|---|
| Sample | Linear Attenuation Coefficient ($cm^{-1}$) | Mass Attenuation Coefficient ($cm^3/g$) | Linear Attenuation Coefficient ($cm^{-1}$) | Mass Attenuation Coefficient ($cm^3/g$) |
| Bran Bread | 0.182 | 0.35436 | 0.158 | 0.307 |
| Mushroom Bread | 0.146 | 0.4252 | 0.113 | 0.329 |
| Multibran Cookies | 0.2 | 0.2954 | 0.225 | 0.262 |
| Mushroom cookies | 0.22 | 0.31186 | 0.175 | 0.2474 |
| Wheat flour cookies | 0.193 | 0.2734 | 0.121 | 0.1714 |

**Table 4.11: Linear Attenuation Coefficient and Mass Attenuation Coefficient of different products**

### 4.7.4. Improvement in shelf life of bread and cookies

In this section, we have discussed about the detected radiation counts in value added baked products (Breads and cookies) and the improvement in shelf life of these products.

| Radiation Counts in Mushroom Bread | | | | | | Radiation Counts in Bran Bread | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| S.N. | Dose(μGy) | Days | | | | Dose(μGy) | Days | | | |
| | | Day1 | Day2 | Day3 | Day4 | | Day1 | Day2 | Day3 | Day4 |
| 1. | 33 | 354 | 223 | 18 | 0 | 46.93 | 292 | 169 | 160 | 0 |
| 2. | 42.36 | 279 | 49 | 0 | 0 | 57.36 | 356 | 147 | 0 | 0 |
| 3. | 64.39 | 171 | 162 | 0 | 0 | 87.99 | 307 | 282 | 0 | 0 |
| 4. | 77.17 | 732 | 506 | 409 | 0 | 163 | 454 | 97 | 0 | 0 |
| 5. | 93.25 | 529 | 196 | 60 | 0 | 234 | 72 | 44 | 0 | 0 |
| 6. | 147.92 | 184 | 10 | 0 | 0 | 250.84 | 466 | 282 | 0 | 0 |

**Table 4. 12: Observed Radiation Counts in Bread Samples**

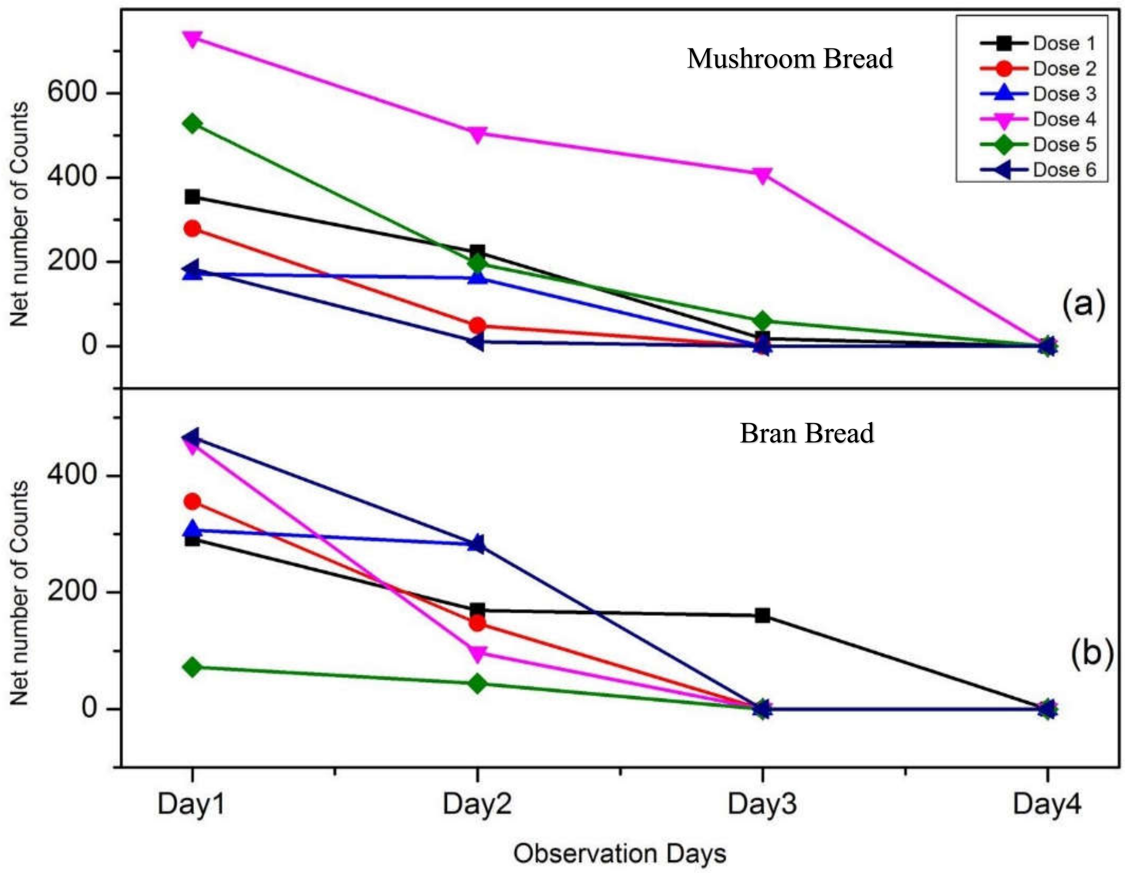


**Figure 4. 13: Graphical Representation of Observed radiation counts in (a) Mushroom Bread and (b) Bran Bread**

| **Radiation Counts in Multibran Cookies** | | | | |
|---|---|---|---|---|
| ***Dose (mGy)*** | ***Day1*** | ***Day2*** | ***Day3*** | ***Day4*** |
| **0.02** | 286 | 151 | 105 | 0 |
| **0.024** | 40 | 36 | 0 | 0 |
| **0.025** | 357 | 77 | 0 | 0 |
| **0.026** | 212 | 104 | 45 | 0 |
| **Radiation Counts in Mushroom cookies** | | | | |
| **0.27** | 383 | 200 | 0 | 0 |
| **0.29** | 383 | 128 | 68 | 0 |
| **0.35** | 137 | 83 | 53 | 0 |
| **0.4** | 340 | 270 | 0 | 0 |
| **Radiation Counts in Wheat-flour cookies** | | | | |
| **0.19** | 343 | 74 | 0 | 0 |
| **0.2** | 509 | 119 | 88 | 0 |
| **0.31** | 511 | 151 | 136 | 0 |
| **0.35** | 364 | 250 | 0 | 0 |

**Table 4. 13: Observed Radiation Counts in formulated Cookies**

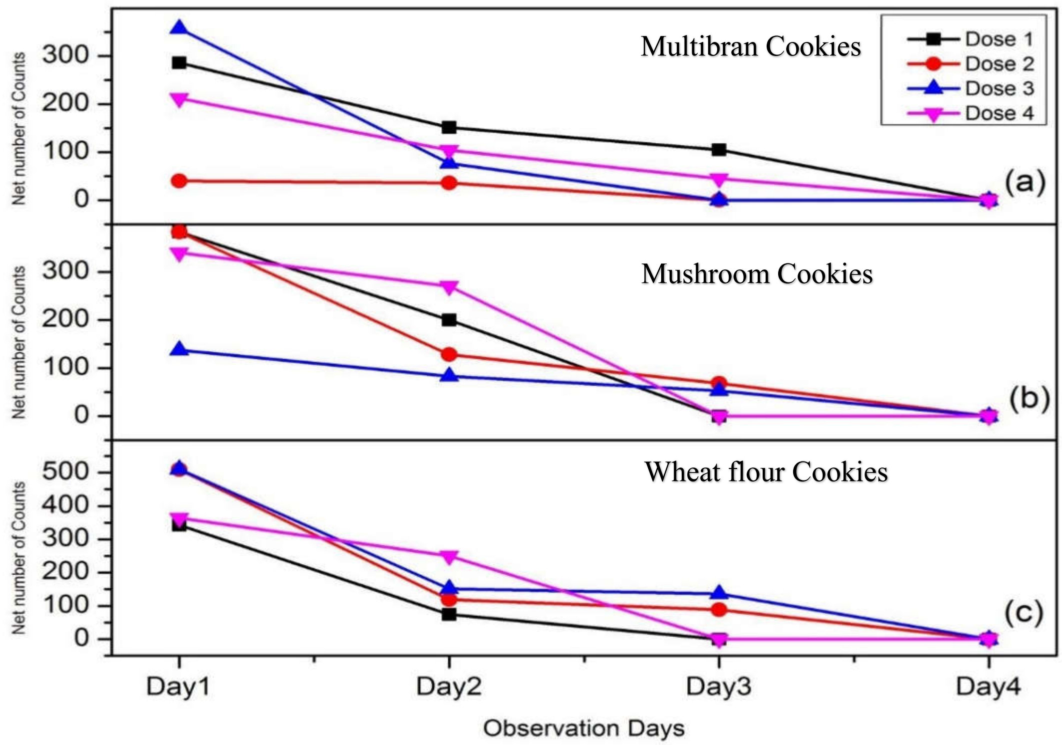


**Figure 4. 14: Graphical Representation of Observed radiation counts in Multibran Cookies, Mushroom cookies, Wheat-flour cookies**

In Table 4.12 & Table 4.13, we have tabulated the radiation counts observed after irradiating bread and cookies samples to the gamma radiations; Observations were taken till the counts by samples and background counts become same. Graphical representation of these counts for individual samples has been shown in Fig. 4.14 and 4.15. All the five samples involving Mushroom Bread, Bran Bread, Multibran Cookies, Mushroom Cookies and Wheat-flour Cookies has been exposed to very low radiation doses, it has been observed that the residual radiations last for 3 days at max in all these food samples which means after three days of irradiating these food products are safe for consumption by human being. Therefore, irradiated food products ensure food safety, reduce losses during transportation and commercialization and satisfy quarantine requirements (Yousefi and Razdari, 2014). Irradiating food samples to very low doses of gamma radiation doesn't cause significant changes in temperature, texture or other sensory properties of the ingredients (Farkas, 2006). It is observed that the irradiated bread samples become a little hard and that may be because of reduction in the size of air bubbles inside the irradiated bread (Ansari *et al.,* 2019).

Irradiated breads showed prolonged shelf life of seven days as compared to the non-irradiated control sample breads for four days. Prolongation of shelf life of irradiated bread samples results because of inhibition in the growth of mold (Box 1959).


**Non-irradiated Bread showed spoilage after 4 days of manufacture date** | **Non-irradiated Bread with spoilage** | **Irradiated Bread**

**Figure 4. 15: Shelf life study of chemical preservative free bread**

Nutrient analysis of the irradiated products had been checked to observe the effect of radiation on chemical composition. Analysis had been done when the dose count of the irradiated products became zero to check the edibility of the products. Even after becoming zero dose count of radiation, products showed visual microbial spoilage few days later. Hence it was necessary to check the quality of the products in between the time period of zero dose count to visual microbial growth.

The current study involves freshly prepared food samples viz. mushroom bread, bran bread, mushroom cookies, multibran cookies, and wheat flour cookies without using any chemical preservatives. According to the estimated nutrient composition, formulated products showed low carbohydrate but higher fibre and protein contents. These products were irradiated using very low doses of gamma sources viz., $^{60}$Co and $^{137}$Cs source to improve shelf life. Evaluated mass attenuation coefficient of these food samples revealed decreasing coefficient value with increase in photon energy. Shelf life of irradiated breads was increased to seven days in comparison with that of non-irradiated breads which was four days. Irradiated cookies showed zero count after 3rd day of exposure.

| **Products (After showing zero count)** | **Moisture (%)** | **Ash (%)** | **Crude fat (%)** | **Crude protein (%)** | **Crude fibre (%)** | **Total carbohydrate (%)** |
|---|---|---|---|---|---|---|
| Bran bread (After 4th day) | 22.78±0.05$^{a}$ | 2.51±0.09$^{b}$ | 2.53±0.23$^{b}$ | 12.24±0.01$^{b}$ | 2.98±0.12$^{b}$ | 56.96±0.65$^{b}$ |
| Multibran Cookies (After 3rd day) | 4.99±0.12$^{b}$ | 6.58±0.05$^{a}$ | 4.53±0.01$^{a}$ | 18.11±0.01$^{a}$ | 4.98±0.14$^{a}$ | 60.81±1.25$^{a}$ |

**Table 4. 14: Nutrient composition of baked food products after irradiation**

| **Products** | **Free fatty acid value (mg KOH/100g)** | **Peroxide value (meq peroxide/1000g)** |
|---|---|---|
| Bran bread (After 4th day) | 0.40±0.12$^{b}$ | 2.31±0.45$^{a}$ |
| Multibran Cookies (After 3rd day) | 0.54±0.32$^{a}$ | 2.26±0.02$^{b}$ |

**Table 4. 15: Chemical composition of food products after irradiation to study storage stability**

The recent study explored a significant way to improve shelf life of nutrient rich products by using gamma radiation instead of chemical preservatives. This study will be helpful to bakery industry to stop food spoilage without use of harmful chemical preservatives.

## 4.8. Summary

We have studied the nutrient composition, organoleptic evaluation and shelf-life of highly nutritive multibran cookies formulated with partial replacement of wheat flour with the milling by-products i.e., chickpea husk, moong bean husk, rice bran, broken rice, and wheat bran using central composite design of response surface methodology. Multi-bran cookies

(MBC) showed enhanced nutritional profile in comparison with the wheat flour cookies (WFC) served as reference sample. MBC showed 18% crude protein, 5% crude fibre, higher than the crude protein (7.78%) and crude fibre (2%) of WFC. However, total sugar concentrations of MBC (3.08 g/100g) was lower than WFC (4.89 g/100g). Calcium and phosphorus present in MBC were 115.06 mg/100g and 195.88 mg/100g respectively, significantly higher ($p < 0.05$) than WFC. The overall acceptability of MBC as indicated by 9-point hedonic scale (8.13) was satisfactory.

To enhance shelf-life of breads and cookies by replacing the conventional preservation techniques to irradiation by gamma radiations of very low dose, in the range of μGy obtained from the radioactive sources like $^{137}Cs$ and $^{60}Co$. Uniquely prepared breads were irradiated to 6 different doses and cookies were irradiated to 4 different doses. It has been observed that food samples have shown enhanced fibre and nutrient contents. Also the shelf life of prepared food sample enhanced significantly.