Formulation and characterization of cookies prepared from the composite flour of germinated kidney bean, chickpea, and wheat

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Abstract
The objective of this study was to optimize the formulation of cookies from the composite flour of germinated kidney bean, chickpea, and wheat using response surface methodology (RSM). Snap force, spread ratio, and overall acceptability served as responses for the optimization of cookie formulation. Optimization and validation of central composite rotatable design (CCRD) of RSM concluded the feasibility of using 19.11 g of germinated kidney bean flour, 31.19 g of germinated chickpea flour, and 50.00 g of germinated wheat flour, per 100 g of flour composition for cookie preparation. Characterization of the novel formulated product was done by analyzing various attributes of flour and cookies. Optimized composite flour formulation (OCFF) exhibited appropriate functional and pasting characteristics required for cookie preparation. Optimized composite flour cookies (OCFCs) had a higher amount of protein (12.32 ± 0.11), fats (22.57 ± 0.23), and crude fiber (5.64 ± 0.02) content as compared with ungerminated wheat flour cookies (UWFCs). In vitro digestibility (carbohydrate and protein) was significantly higher in OCFCs owing to the utilization of germinated grain’s flour. Amino acid content of germinated grains enhanced the total essential amino acids in OCFCs. Shelf life of the formulated product was acceptable for up to 90 days when stored at 25°C in aluminum-laminated sealed bags.

KEYWORDS
composite flour, cookies, germination, in vitro digestibility

1 | INTRODUCTION

Wheat flour is an ideal ingredient for various food product formulations; therefore, it is widely used for the production of bakery and confectionery products (Diana, Mirela, & Jianu, 2007). The suitability of wheat flour in bakery and confectionery products could be attributed to its gluten content. Out of few problems associated with the use of wheat, scarcity of wheat in certain regions and gluten-related allergies are common.

Diet-related chronic disease and malnutrition give rise to the concept of novel food processing techniques, of which composite flour is one of the effective solutions. Composite flour enriched with functional components like tubers, starches, legumes, cereals (Bourré et al., 2019; Noorfarazhizah et al., 2014), and multigrain premixes (Kumar, Sharma, Khan, Govindaraj, & Semwal, 2015) reduced the risk of diet-related disease and allergies (Adebowale, Adegoke, Sanni, Adegunwa, & Fetuga, 2012; Mepba, Eboh, & Nwaoijigwa, 2005). Various researchers found the suitability of composite flour for the...
preparation of bakery products (Gómez, Oliete, Rosell, Pando, & Fernandez, 2008; Ribotta, Arnulphi, León, & Anón, 2005). Recent trend has also shown the utilization of processed flours or components that could enhance the nutritional quality of a product. Processing of flour may include germination of grains (Nkhata, Ayua, Kamau, & Shingiro, 2018), fortification (Stabnikova, Antoniuk, Stabnikov, & Arsen'eva, 2019), and fermentation (Bourré et al., 2019) of cereal or legumes. Germination is one of the best and inexpensive methodologies to enhance the nutritional quality and functional characteristics of the grains. Germination allows predigesting of grain’s components and enhances free amino acids, vitamins, and functional characteristics (Hallén, Ibanoglu, & Ainsworth, 2004).

In the present research, supplementation with germinated legume flours was adopted to formulate a product with high nutrition and digestibility. Final formulation was characterized for its nutritional, in vitro digestibility, amino acid profile, texture profile, and shelf life.

2 | MATERIALS AND METHODS

2.1 | Raw material

Wheat (PBW-550), kidney bean (light speckled kidney beans), and chickpea GPF-2 (GF-89-36) were procured from certified seed agency. Selected grains were germinated as per the method described by Arora, Jood, Khetarpaul, and Goyal (2009). The sprouts were rinsed in water after germination, dried initially at 80°C for 15 min to arrest the enzyme activity, and then finally dried at 55–60°C to moisture content of 8.00 ± 1% (db). Dried sprouts and ungerminated wheat grains (PBW-550) were ground separately in lab grinder to fine powder in the form of flour and passed through 60 mesh sieves (US size 60 mesh = 250 μm).

2.2 | Experimental design

2.2.1 | Selection of independent variables and responses

Selected germinated legume flours other than germinated wheat flour were selected as independent variables. Response surface methodology (RSM) was used as optimization technique, which has been proven as appropriate tool in the optimization of various bakery products like sponge cakes (Chaiya, Pongsawatmanit, & Prin-yawiwatkul, 2014), gluten-free bread (Sanchez, Osella, & Torre, 2004), cassava cake (Gan et al., 2007), and chocolate cake (Moscatto, Borsato, Bona, Oliveira, & Hauly, 2006). Responses like spread ratio, snap force, and overall acceptability were selected by putting a hypothesis that with the incorporation of germinated grains, these functions could be related to specific composition to fit the regression equation, which describes the quality composition responses. To serve the purpose of optimization and to evaluate the effect of selected variables on the responses, central composite rotatable design (CCRD) was chosen. Preliminary studies have shown that replacing wheat more than 50% with germinated legumes was not feasible for production of cookies from composite flour containing germinated legumes. Based on trial runs, the upper and lower range for germinated kidney bean flour was selected between 15% and 25%, whereas the range for germinated chickpea flour was kept between 25% and 35%. CCRD was segregated into three different parts, that is, factorial design (two levels), axial points (outside core), and center points. RSM statistical software (design expert 10) distributed the design into 13 experiments with four factorial points, five replicates (center points), and four axial points. The CCRD variables and responses for different formulations are as shown in Table 1. Experiments were carried out in random order to minimize the effect of unexplained variability owing to extraneous factors (Myers & Montgomery, 2002).

Result of responses was analyzed using regression analysis by fitting a suitable model, that is, second-order polynomial equation:

\[ Y = \beta_0 + \sum_{i=1}^{n} \beta_i x_i + \sum_{i=1}^{n} \beta_i x_i^2 + \sum_{i<j=1}^{n} \beta_{ij} x_i x_j, \]

where \( \beta_0 \) is the value of response at the center points (0, 0); \( x_i \) and \( x_j \) are the variables of design; \( \beta_i, \beta_{ij}, \) and \( \beta_{ij} \) are regression coefficients; and \( n \) is the number of variables.

2.2.2 | Preparation of cookies

Cookies were prepared using a traditional method as described by Chauhan, Saxena, and Singh (2015). Ingredients used were composite flour (proportions were kept according to values given by experimental design), 100 g; ground sugar, 40 g; sodium bicarbonate, 1.0 g; sodium chloride, 1.0 g; bakery shortening, 50 g; skim milk powder, 20 g; and water, 20 ml. A premix of flour, milk powder, and sodium bicarbonate was prepared separately. Dough prepared was formed into sheet of approximately 0.5-cm thickness, and then, a circular mold was used to cut the dough sheets. Baking was carried out in baking oven (conventional baking oven, Continental India) at 170°C for around 15 min. After 15 min, cookies were allowed to cool down at room temperature. Cookies were packed in aluminum-laminated sealed bags for further analysis.

2.2.3 | Data analysis and estimation of responses

Regression analysis of the experiments was carried out by stepwise variable selection and backward elimination techniques. Numerical technique and mapping of responses were used for maximization and minimization of polynomial fitting. This was performed by the use of Stat-Ease Design expert software (Ver. 10.0) developed by Stat-Ease Inc., Minneapolis. The response surfaces and three-dimensional (3D) contour graphs were plotted using two function variables. All variables with significant levels of \( p \leq 0.05 \) were included in a model, and...
coefficient of determination was evaluated ($R^2$) to observe the accuracy of the model. Samples were estimated for quality characteristics like snap force (N), spread ratio, and overall sensory attributes. Values reported were average of triplicate determinations.

2.2.4 | Spread ratio

Spread ratio was measured as ratio of diameter to thickness of the cookies (Zoulias, Piknis, & Oreopoulou, 2000). Average value of triplicate measurements was reported, and ratio was observed as

\[
\text{Spread ratio} = \frac{\text{Diameter of cookie (mm)}}{\text{Thickness of cookie (mm)}}.
\]

2.2.5 | Snap force

A three-point bend test was used to estimate the snap force required to break down the cookie (Chauhan et al., 2015). For this purpose, TA.XT2i Stable Micro System texture profile analyzer (Stable microsystem, England) was used with three-point bend rig arrangements. Mode of measurement was in compression with units in Newton (N). The measurements were carried out under following test conditions. Pretest speed was 1.5 mm/s, test speed was kept at 2.0 mm/s, posttest speeds were kept at 10.0 mm/s, distance of probe was maintained at 30 mm, automatic trigger type force was 20 g, and method settings were maintained by adjusting data acquisition rate at 200 pps. Graph was observed between force (N) and time (T). The peak force required to break down the cookies was reported as snap force (Sindhuja, Sudha, & Rahim, 2005). Results were observed in triplicates.

2.2.6 | Overall acceptability

A descriptive sensory analysis was carried out by preparing the chart and setting out the sensory characteristics as per ISO 13299:2016 procedure, using semitrained analysts to observe the sensory score of cookies. Overall acceptability was reported finally by calculating the average of reported sensory attributes (Gajera, Kapopara, & Patel, 2010).

2.3 | Characterization of formulated bakery product

2.3.1 | Functional properties of flours

Functional properties of flours were observed by analyzing the water absorption capacity, oil absorption capacity, sedimentation value, foaming capacity, and emulsification capacity. Water absorption capacity was done by the method of Yamazaki (1953). Oil absorption capacity was estimated by the method of Lin, Humbert, and Sosulski (1974). Sedimentation value was estimated by calculating as the swelling power of flour by using ICC 116/1 standard method (Zeleny’s method). Foaming capacity (%) was estimated as per the method described by Mizubuti, Junior, Souza, da Silva, and Ida (2000). Emulsification capacity was calculated by the method of Naczk, Diosady, and Rubin (1985). The emulsification capacity (EC) was expressed as ml of oil emulsified by 1.0 g of the sample.

2.3.2 | Pasting properties of flours

Pasting properties of different flours were estimated using Rapid Visco Analyzer (RVA Tecmaster, Perten, Australia), using standard testing profile-1. Appropriate sample around 5 g as described in

| Experiment no. | Variables (coded) | Variables (actual) | Responses |
|---------------|-------------------|-------------------|-----------|
|               | $x_1$ ($x_1$)    | $x_2$ ($x_2$)    | $Y_1$ ($Y_1$) | $Y_2$ ($Y_2$) | $Y_3$ ($Y_3$) |
| 1             | 1.00              | 1.00              | 25.00      | 35.00      | 6.20       | 51.24     | 4.22      |
| 2             | −1.414            | 0.00              | 12.93      | 30.00      | 6.30       | 38.74     | 3.93      |
| 3             | 0.00              | 0.00              | 20.00      | 30.00      | 6.40       | 42.18     | 4.10      |
| 4             | −1.00             | 1.00              | 15.00      | 35.00      | 6.30       | 43.74     | 3.93      |
| 5             | 1.00              | −1.00             | 25.00      | 25.00      | 6.13       | 56.17     | 3.85      |
| 6             | 0.00              | 0.00              | 20.00      | 30.00      | 6.35       | 42.12     | 3.90      |
| 7             | 1.414             | 0.00              | 27.07      | 30.00      | 6.16       | 48.26     | 3.75      |
| 8             | −1.00             | −1.00             | 15.00      | 25.00      | 6.08       | 48.15     | 4.22      |
| 9             | 0.00              | 0.00              | 20.00      | 30.00      | 6.42       | 45.76     | 3.92      |
| 10            | 0.00              | 0.00              | 20.00      | 30.00      | 6.38       | 43.50     | 4.05      |
| 11            | 0.00              | 0.00              | 20.00      | 30.00      | 6.40       | 41.36     | 3.98      |
| 12            | 0.00              | 1.414             | 20.00      | 37.07      | 6.21       | 58.03     | 4.75      |
| 13            | 0.00              | −1.414            | 20.00      | 22.93      | 6.05       | 69.88     | 4.73      |

Note: $x_1$, kidney bean; $x_2$, chickpea; $Y_1$, spread ratio; $Y_2$, snap force; $Y_3$, overall acceptability.
manual was taken and mixed with 25 ml of water. The mixture was stirred for 10 s at 960 and then 160 rpm. Initially, temp–time combination of 50°C for 1 min was employed, and then, after equilibrium, temperature raised to 95°C for 5 min. After the procedure, cooling cycle was carried out by decreasing temperature to 50°C in 3 min. Results were recorded from graph as peak viscosity (PV), trough viscosity (TV), breakdown value, final viscosity (FV), setback, and pasting temperature (PT).

2.3.3 | Proximate analysis and in vitro digestibility of cookies

Moisture content, crude fat content, crude fiber, and ash content of cookies prepared from optimized formulation were analyzed using standard AOAC methods (AOAC, 2005). Total carbohydrate content was estimated by anthrone method (Ludwig & Goldberg, 1956). Protein content of cookies was estimated by the method of Lowry, Rosebrough, Farr, and Randall (1951). To evaluate the protein digestibility, in vitro method suggested by Chavan, Chavan, and Kadam (1988) was used. This method is based on the digestion of sample by pepsin and pancreatic enzymes. Digested and nondigested sample were analyzed for protein by using method of Lowry et al. (1951). In vitro protein digestibility was calculated as proportion of protein digested using following relationship:

\[
\text{In vitro protein digestibility (\%)} = \frac{\text{Total protein} - \text{Residual protein}}{\text{Total protein}} \times 100.
\]

In vitro carbohydrate digestibility was analyzed using the modified method of Modi and Kulkarni (1976). Preweighted defatted sample of cookies was treated with 1.5 ml of amylglucosidase enzyme prepared in a 50 mM of sodium acetate buffer (pH 4.5) and incubated at 37°C for 2 h. After incubation, solution was heated to stop enzymatic reactions. In vitro carbohydrate digestibility was calculated as a proportion of residual carbohydrate to total carbohydrates and expressed as percentage.

\[
\text{IVCD (\%)} = \frac{\text{Total carbohydrate} - \text{Residual carbohydrate}}{\text{Total carbohydrate}} \times 100.
\]

2.3.4 | Texture profile analysis of cookies

The three-point bend test was performed by using TA.XT2i Stable Micro System texture profile analyzer (Stable Microsystems, England) to estimate the texture parameters (hardness, fracturability, and cohesiveness) of optimized cookies with similar settings as performed for snap test (as discussed in Section 2.2.5). Hardness and cohesiveness were estimated from force to deformation curve of compression test (Meullent & Gross, 2007).

2.3.5 | Color characteristics of cookies

Color value of different cookies was estimated by using Hunter Lab (HunterLab digital colorimeter, D25M, Reston, Virginia) equipped with I-type optical sensor (D-25) and DP-9000 processor. \( L^* \) depicts lightness, \( a^* \) indicates redness (+)/greenness (–), and \( b^* \) indicates yellowish (+)/blueness (–) of the sample. Values of sample were compared with the standard colored reference tile \( (L_0 = 25.54, a_0 = 28.89, \text{ and } b_0 = 12.03) \). Cookie samples were placed on specimen port, and the values of \( L^* \), \( a^* \), and \( b^* \) were recorded.

2.3.6 | Amino acid content of cookies

Amino acid content of cookies was analyzed using the physiological kits of gas chromatography–flame ionization detection (Phenomenex, USA). Grounded samples were defatted and then hydrolyzed with concentrated HCl. Analysis was performed as instructed in the kit’s manual. The GC column used was the ZB-AAA-GC column, which was provided in the kits, and standard analysis conditions were used, as described in the kit’s manual. The results obtained were expressed as amino acid (g/16 g N).

2.3.7 | Storage studies of cookies

For storage studies, both types of cookies were packed separately in aluminum-laminated sealed bags and stored at 25°C. Different packs of cookie sample (containing 20 cookies each) were prepared for different days (0, 10th, 20th, ..., 90th days) of analysis and were labeled accordingly. Microbiological analysis (total plate count) of sample was done according to standard AOAC method (AOAC, 2005) on priority basis after opening the packaging, to avoid contamination. Moisture content, peroxide value, and free fatty acid value of cookies were also analyzed as per the methods of AOCS (1990). Sensory analysis of cookies was performed at regular intervals to observe the effect of storage time on cookies.

3 | RESULTS AND DISCUSSION

3.1 | Diagnostic checking of fitted model

After model reductions, coefficients of regression were observed. Significance of each model was examined by analysis of variance for observed responses. Model equations were analyzed to observe the linear and quadratic effects of variables. Interaction of variable and their effect were observed through regression equations after model reduction. Multiple regression equations at significant levels related to
responses such as spread ratio, snap force, and overall acceptability were reported as follows:

\[
\text{Spread ratio } (Y_1) = 6.3882 - 0.0303x_1 + 0.0646x_2 - 0.0803x_1^2 - 0.1298x_2^2 - 0.0376x_1x_2 \left( R^2 = 0.969 \right).
\]

\[
\text{Snap force } (Y_2) = 42.9840 + 3.6234x_1 - 3.2627x_2 - 0.7174x_1^2 + 9.5095x_2^2 - 0.1298x_1x_2 \left( R^2 = 0.944 \right),
\]

\[
\text{Overall acceptability } (Y_3) = 3.9890 - 0.0409x_1 - 0.0132x_2 - 0.1339x_1^2 + 0.3161x_2^2 + 0.1675x_1x_2 \left( R^2 = 0.8808 \right).
\]

Values for \( R^2 \) for each model were found valid, which validated the model fitting. Lack of fit, levels of significance, and analysis of variance further suggested the fitting and adequacy of model up to a desirable significant level. Linear and quadratic effects of the independent variable could be estimated from the regression equations after model reduction. Negative linear effect of germinated kidney bean was observed on spread ratio and overall acceptability, whereas positive effect was observed on snap force. Germinated chickpea showed a positive linear effect on spread ratio, whereas negative effect on snap force and overall acceptability was observed. Quadratic effect of germinated kidney bean was observed negative for all responses. Germinated chickpea exhibited negative quadratic effect on spread ratio, and positive quadratic effect was observed for snap force and overall acceptability. Interaction terms of both variables showed significant negative effect on spread ratio and snap force, whereas positive interaction was observed for overall acceptability. Analysis of variance was observed for the regression models to observe the variation in responses at different levels of substitution. Regression, lack of fit, pure error, and residual values were observed as shown in Table 2. \( F \) test was conducted to check the adequacy of model. The model \( F \) values of spread ratio, snap force, and overall acceptability imply that the model was significant, and there was less chance that a model \( F \) value could occur this large owing to noise. Values of “Prob > \( F \)” less than 0.05 indicated that model terms were significant for each response. Lack of fit was nonsignificant; therefore, the model was adequately fit. Regression value of spread ratio and overall acceptability was low; therefore, the residual values were closer to 0.

### 3.2 Analysis of variables and responses

Three-dimensional plots were formed by the responses obtained after experimental values in the given set of design. Three-surfaced graph provides the predicted data at a very small level of variations among independent variables. Spread ratio increased with the increase in the germinated chickpea proportion in cookies, which could also be attributed to the positive linear effect on spread ratio (Figure 1a). A lower increment in the spread ratio was observed from the plot when germinated kidney bean proportion was increased.

Snap force dropped at different levels of chickpea substitution, whereas addition of germinated kidney bean caused an increment in the hardness of cookies (Figure 1b). Axial points had higher values of

| TABLE 2 | Analysis of variance of values for responses for the level of significance for optimization of variable and responses of formulation |
|-----------------|-------------------------------------------------------------------------------------------------------------------------------------|
| **Source of variation** | **Sum of squares** | **Degree of freedom** | **Mean square** | **\( F \) value** |
| **Y₁ (spread ratio)** | Regression | 0.1920 | 5 | 0.0384 | 45.10*** |
| | Lack of fit | 0.0033 | 3 | 0.0011 | 0.308 NS |
| | Pure error | 0.0026 | 4 | 0.0007 | NS |
| | Residual | 0.0060 | 7 | 0.0009 | |
| | Total | 0.1980 | 12 | |
| | Adeg. precision | 17.7079 | |
| **Y₂ (snap force)** | Regression | 846.4740 | 5 | 169.29 | 23.76*** |
| | Lack of fit | 37.8809 | 3 | 12.626 | 4.20 NS |
| | Pure error | 12.0027 | 4 | 3.001 | NS |
| | Residual | 49.8837 | 7 | 7.126 | |
| | Total | 896.3577 | 12 | |
| | Adeg. precision | 16.6480 | |
| **Y₃ (overall acceptability)** | Regression | 1.0392 | 5 | 0.208 | 10.341*** |
| | Lack of fit | 0.1118 | 3 | 0.037 | 5.153 NS |
| | Pure error | 0.0289 | 4 | 0.007 | NS |
| | Residual | 0.1407 | 7 | 0.020 | |
| | Total | 1.1799 | 12 | |
| | Adeg. precision | 10.1390 | |

Abbreviation: NS, nonsignificant.

* \( p < 0.1 \)

** \( p < 0.05 \)

*** \( p < 0.01 \)
hardness as compared with values closer to the center points. Therefore, the quadratic effect of interaction of both flours was in negative correlation.

Hyperbolic paraboloid 3D graph of overall acceptability was observed as shown in Figure 1c. The increase in the germinated legume substitution actually lowered the overall acceptability of cookies, but overall acceptability within range for the product acceptability could be formed by interacting the two flours near to the saddle points. The orthogonal function of equation provided the minimax point in between two slopes, relative to both proportions. Axial points delivered the high acceptance values, whereas central point provides the saddle point owing to interaction of both independent variables. Therefore, as a result, interaction of both variables gave a positive correlation and positive effect on overall acceptability.

3.3 | Optimization of responses

Model equations were reduced to evaluate the effect of variabilities of responses with respect to the independent variables. Maximization of desirable responses was required to optimize the final product formulations. Within range, independent variables were selected by the design expert software to formulate the product with optimum responses and a novel product formulation. Design expert provided the valid and within range set of solutions. Selected solutions were analyzed to obtain the actual responses in the available set of conditions and then were compared with predicted values for validation purpose. A lower set of deviations from predicted and experimental actual values was selected as optimal outcome of design. The RSM-based CCRD design was finally obtained for independent variables with flour formulations of 31.19 g of chickpea and 19.11 g of kidney bean with optimum responses. Predicted responses were found close and actual responses, which were obtained as 6.41 for spread ratio 41.98 N for snap force and overall acceptability of 4- on 5-point scale.

3.4 | Characterization of optimized composite flour and cookies from optimized formulation

3.4.1 | Functional and pasting formulation of ungerminated wheat flour and OCFF

Functional properties like water absorption capacity, oil absorption capacity, sedimentation value, foaming capacity, and emulsification capacity were observed as reported in Table 3. Germination resulted in the increased protein content of flours; however, the resultant protein content due to legume flour also attributed to variation in the functional and pasting properties. These properties are a type of biophysical properties, which reveals the interaction of molecules with the ingredients and thus provides the formal idea of flour behavior for a product formulation. Legume-blended flour (optimized composite flour formulation [OCFF]) exhibited higher water absorption capacity (1.46 ± 0.04) than did ungerminated wheat flour (UWF; 1.33 ± 0.02). Germination enhanced the disruption of polysaccharides that led to
more damaged starch and thus retained more water. Another reason could be attributed to higher protein content in legumes, which tended to absorb more water (Chauhan et al., 2015). Oil absorption capacity of OCFF was also observed higher than in UWF. The observed values for oil absorption capacity of OCFF were 1.38 ± 0.03 g/g, whereas UWF was reported to have 1.16 ± 0.02 g/g of oil absorbed. The increase in the protein content and hydrophobic interaction of resultant protein molecules of blended flours could be attributed to the variation in oil absorption capacities (Chiemela, Olufemi, & Joseph, 2009). Sedimentation value of raw wheat flour was 60.01 ± 0.01 ml. With blending of different flours and germination, there was reduction in the total gluten fraction that led to a lowered value of sedimentation. Germination and blending of flour affected the foaming capacity of composite flour. Foaming capacity of OCFF (21.43 ± 0.03) was reported than that of UWF (12.76 ± 0.01). The variation in the emulsification capacities could be attributed to the protein–lipid interactions (Millward & Rivers, 1988; Sibian et al., 2017).

Rheology of blended doughs was compared by using RVA, to observe the pasting properties related to flours. RVA gave multi-parameter values like peak viscosity, trough viscosity, breakdown, final viscosity, setback viscosity, and pasting temperature to observe the behavior of viscosity-related molecules (Table 3). Owing to germination, there was degradation of starch molecule, which leads to lower value of peak viscosity in OCFF. Peak viscosity (cP) of UWF was observed to be 928.00 ± 0.34, which was higher than that of OCFF (519.00 ± 0.47). Trough viscosity is the measure of extreme attainable viscosity at given temperature. Owing to degradation in starch, the trough viscosity of OCFF was reported to be lower (495.00 ± 0.38 cP). Breakdown determines the thermal and pasting stability of flour. Owing to germination and substitution of legumes flour, the ability of pasting and thermal stability were higher in OCFF. The observed values for breakdown in OCFF and UWF were 24.00 ± 0.43 and 67.00 ± 0.51 cP, respectively. Final viscosity is the index of flour’s ability to form viscous paste. Final viscosity of ungerminated flour (1,124.00 ± 0.43 cP) was quite high as compared with that of blended flours (934.00 ± 0.44 cP). Higher retrogradation tendency and higher setback viscosity was shown by OCFF (439.00 ± 0.52 cP). Lower retrogradation value leads to slow rate of staling process of product. UWF was observed with low setback viscosity (263.00 ± 0.38 cP). Pasting temperature correlates to gelatinization tendency of flour. Owing to degradation of starch, gelatinization tendency of legume-blended OCFF was lower. Peak time provides the information for total time required for cooking of flour. Blending of flours with different characteristics allowed the interaction of molecules; therefore, the difference in cooking time was obtained as a result (Adebowale et al., 2012).

### 3.4.2 | Proximate analysis and in vitro digestibility of cookies

Table 4 indicates the difference in compositional characteristics of optimized composite flour cookies (OCFCs) and UWF cookies (UWFCs). Changes in the proximate composition of flours were previously reported in wheat and chickpea (Sibian et al., 2017; Sibian, Saxena, & Riar, 2016).
TABLE 4 Compositional analysis and in vitro digestibility of cookies from optimized composite formulation and ungerminated wheat

|                      | Ungerminated wheat flour cookies (UWFCs) | Optimized composite flour cookies (OCFCs) |
|----------------------|----------------------------------------|----------------------------------------|
| Moisture             | 3.57 ± 0.01a                           | 4.89 ± 0.01a                           |
| Protein              | 7.84 ± 0.02a                           | 12.32 ± 0.11a                          |
| Carbohydrates        | 69.75 ± 0.47a                          | 54.43 ± 0.42a                          |
| Fats                 | 18.34 ± 0.26b                          | 22.57 ± 0.23a                          |
| Crude fiber          | 1.93 ± 0.03a                           | 5.64 ± 0.02a                           |
| Ash                  | 0.82 ± 0.02b                           | 1.43 ± 0.02a                           |
| In vitro carbohydrate digestibility | 38.67 ± 0.03b | 50.27 ± 0.06a |
| In vitro protein digestibility | 51.54 ± 0.05b | 67.53 ± 0.04a |

Note: n = 3. Results are expressed in percentage and as mean values ± standard deviations. Means in a column with different subscripts are significantly different (p < 0.05).

Moisture content was higher in OCFCs (4.89 ± 0.01) owing to higher protein content of composite flour. With the substitution of germinated flour of grains, the protein content of OCFCs (12.32 ± 0.11) improved significantly. OCFCs tended to retain more oil during baking than did UWFCs (18.34 ± 0.26), which resulted in higher fat content in OCFCs (22.57 ± 0.23). Elin and Paul (2004) observed the changes during substitution of wheat flour biscuits with germinated chickpea as compared with control sample containing 10% chickpea control. Ash content was also higher in OCFCs (1.43 ± 0.02), whereas lower values were reported in UWFCs (0.82 ± 0.02). Crude fiber content of OCFCs was quite higher and differed significantly with that of the cookies prepared from UWF. Germination increases the crude fiber content of grains owing to cell wall polysaccharides, and therefore, more fiber content was observed in OCFCs (5.64 ± 0.02). in vitro digestibility studies had shown the lower protein (51.54 ± 0.05) and carbohydrate digestibility (38.67 ± 0.03) in UWFCs. The higher in vitro protein (67.53 ± 0.04) and carbohydrate digestibility (50.27 ± 0.06) of OCFCs could be attributed to the presence of free amino acid and breakdown of carbohydrate by the action of enzyme alpha amylase during germination. Alonso, Aguirre, and Marzo (2000) conveyed that the in vitro protein digestibility of the Phaseolus vulgaris augmented to approximately 10% as a result of germination.

3.4.3 | Texture profile analysis and color characteristics

Texture profile of cookies was analyzed for the components like hardness, fracturability, and cohesiveness (Table 5). Hardness of UWFCs was reported 62.71 ± 0.05, which was higher than that of OCFCs. A low level of gluten content due to substitution of legume flour caused the arrested gluten matrix, which affected the hardness of cookies (Chauhan, Saxena, & Singh, 2016). Drakos, Andrioti-Petropoulou, Evageliou, and Mandala (2019) observed that biscuits prepared from composite flour have softer and darker appearance than has the control sample. Sindhuja et al. (2005) observed a similar pattern of hardness in composite flour cookies, by replacing wheat with 25–30% amaranth flour. Similar factors that influenced the hardness of cookies also affected the fracturability and cohesiveness of cookies. Fracturability of cookies varied in the range of 7.134 ± 0.03 and 5.785 ± 0.03, with the highest value observed in UWFCs. A higher cohesion value of UWFCs (0.724 ± 0.04) corresponded to their higher break strength, which could be affected by loss of intermolecular attractions (Karaoglu & Kotancilar, 2009). High protein and moisture content could be the factors responsible for the lower cohesion (0.587 ± 0.01) in OCFCs.

Composite flour cookies were slightly darker than wheat flour cookies (Table 5). Color is one of the quality characteristics, which reflects the cookie acceptability by the consumer. Color characteristics reflect the starch dextrinization, caramelization, and Maillard reaction, which are induced by cooking of product (Chung, Cho, & Lim, 2014). Lightness of OCFCs was observed lower as compared with that of UWFCs, which ranged from 52.37 ± 0.04 to 67.54 ± 0.07. The observations are in accordance with the results obtained by Bolarinwa, Lim, and Muhammad (2018) in gluten-free cookies prepared from germinated brown rice. Significant difference was observed in the redness and yellowness of composite flour cookies and UWFCs. Maillard reaction could be accountable for the increased values of a’ and b’ (Islam, Taneya, Shams-Ud-Din, Syduzzaman, & Hoque, 2012) in OCFCs owing to a higher amount of free amino acids and sugars.
3.4.4 | Amino acid profile

A higher amino acid content was observed in cookies prepared from an optimized formulation than in UWFCs. Essential amino acids (UWFCs/OCFC-g/100 g protein) like arginine (2.58 ± 0.01/2.66 ± 0.02), histidine (0.92 ± 0.03/0.95 ± 0.02), leucine (3.41 ± 0.03/5.60 ± 0.04), lysine (0.97 ± 0.04/2.89 ± 0.03), methionine (0.90 ± 0.02/0.95 ± 0.04), phenylalanine (2.06 ± 0.02/2.32 ± 0.06), threonine (1.68 ± 0.03/1.96 ± 0.03), tryptophan (0.57 ± 0.03/0.70 ± 0.01), and valine (2.98 ± 0.02/3.22 ± 0.01) were reported significantly higher in OCFCs, whereas isoleucine (2.52 ± 0.02/2.50 ± 0.06) was slightly higher in UWFCs. Legumes are generally deficient in methionine and correspondingly cereal lacks lysine, but with the processing (germination and substitution) of flour, the formulated cookies were observed with good methionine and improved lysine content. The reason for the good nutritional attribute of legume-blended cookies could be attributed to the amino acid profile of legume flours (Imran et al., 2011). Table 6 represents the total amino acid profile of UWFCs and OCFCs. Total aromatic amino acid content (%) was found lower in OCFCs (8.27 ± 0.04) as compared with UWFCs (8.52 ± 0.06). Aromatic amino acid is more readily available for utilization in chemical changes during baking (Oupadissakoon & Young, 1984). Composite flour thus proved to be beneficial, owing to the supplementation of deficient essential amino acids (Ikumola, Otutu, & Oluniran, 2017).

3.4.5 | Storage studies

Multiple factors like moisture content, peroxide value, free fatty acids, and overall sensory score were observed for the storage studies of cookies (Table 7). Moisture content of cookies sample increased slightly during storage. With the increase in the moisture content, other parameters like peroxide value, free fatty acid, and total plate count increased to some extent. The increase in the moisture content could be attributed to the hygroscopic nature of cookies. The reason for legumes blended cookies to absorb high moisture could also be attributed to higher content of protein and crude fibers. Nagi, Kaur, Dar, and Sharma (2012) reported similar findings in cereal bran-fortified cookies at the end of 90 days. Peroxide value indicates the initial rancidity and degree to which lipid undergoes primary oxidation. Owing to higher moisture and fat content of OCFCs, the peroxide value was also reported to be higher. Cereals contain a lower amount of unsaturated fats; therefore, a lower amount of oxidation took place in UWFCs (2.12 ± 0.01 to 5.16 ± 0.03). Peroxide value of OCFCs was higher even during the initial day of study (4.12 ± 0.04), which increased to 8.53 ± 0.03 mEq/kg on the 90th day. The results corroborated the studies by Divyashree (2014) in buckwheat–chia seed-fortified cookies, which occurred owing to auto oxidation during storage. Ease of oxidation depends on the amount of unsaturated fats, storage conditions, and antioxidant activity of food (Akhter, Haider, Muzamil, Zia, & Salahuddin, 2016). Free fatty acid content was higher in OCFCs at initial day (0.41 ± 0.03) owing to its lipid and moisture content. During the storage, the increase in free fatty acid content of both OCFCs and UWFCs was observed. The values of free fatty acid (mg KOH/g) at the 90th day for UWFCs and OCFCs were observed as 0.68 ± 0.05 and 0.89 ± 0.02, respectively. A similar trend of free fatty acids was also reported in soy-fortified cookies (Singh, Singh, & Chauhan, 2000) and composite pasta (Yadav, Sharma, Chikara, Anand, & Bansal, 2014) with the increase in the storage time. Availability of moisture and other nutritive components promote the growth of microbes in cookies. A higher moisture content was observed in OCFCs, which increased during storage. In addition, composite flour cookies were reported to be high in nutrients, which facilitated the microbial growth. Germination converts the complex molecules into simpler molecules like sugar and amino acids, which might be readily available to the microorganism. Therefore, as a result, the microbial load of cookies increased. According to Gilbert (2000), the permissible limit for total aerobic count of ready-to-eat foods should be less than 10⁶–10⁸ cfu/g. The microbial count of UWFCs was reported in the range of 1.5 × 10⁵ to 1.03 × 10² cfu/g during the 90-day storage period. The initial microbial count in OCFCs was 2.0 × 10² and increased up to 1.07 × 10² on the 90th day of storage. Although there was an increment in microbial count, the total plate count during the storage period of preservation did not exceed the permissible limit. Yusufu, Netala, and Opega (2016) also observed an increase in the total viable count (cfu/g) in composite flour cookies prepared from maize, yam bean, and plantain during storage. Organoleptic properties depend on various intrinsic and extrinsic factors (Mudgil, Barak, & Khatkar, 2017). Sensory evaluation showed that the optimized formulated cookies were acceptable even after the storage period of 90 days. Results for legume-substituted cookies were in agreement with the studies of Noor Aziah, Mohamad Noor, and Ho (2012) on the organoleptic properties of cookies fortified with legume.

TABLE 6 Amino acid profile of cookies from optimized composite flour formulation and ungerminated wheat

|                        | Ungerminated wheat flour cookies (UWFCs) | Optimized composite flour cookies (OCFCs) |
|------------------------|----------------------------------------|-----------------------------------------|
| Total nonessential amino acid | 53.31 ± 0.04a                          | 36.88 ± 0.03b                          |
| Total essential amino acid   | 46.69 ± 0.06b                          | 63.12 ± 0.05a                          |
| Total aromatic amino acid   | 8.52 ± 0.06a                           | 8.27 ± 0.04a                           |
| Total basic amino acid      | 11.07 ± 0.03a                          | 20.32 ± 0.04a                          |
| Total acidic amino acid     | 30.42 ± 0.07a                          | 19.62 ± 0.05b                          |
| Sulfur containing amino acid| 3.86 ± 0.03ab                          | 3.85 ± 0.05b                           |

Note: n = 3. Results are expressed in percentage and as mean values ± standard deviations. Means in columns with different subscripts are significantly different (p < 0.05).
TABLE 7 Partial shelf life studies of cookies from optimized formulation and ungerminated wheat

| Number of days | Moisture content (mEq/kg) | Peroxide values (mEq/kg) | Free fatty acid content (mg KOH/g) | Microbial count (cfu/g) | Overall sensory score |
|---------------|---------------------------|--------------------------|-----------------------------------|------------------------|----------------------|
|               | UWFCs                     | OCFCs                    | UWFCs                             | OCFCs                  | UWFCs                             | OCFCs                   |
| 0             | 3.86 ± 0.02               | 5.12 ± 0.01              | 2.12 ± 0.01                       | 4.12 ± 0.04            | 0.29 ± 0.01                       | 0.41 ± 0.03             | 1.5 × 10³ ± 0.07 | 2.0 × 10¹ ± 0.03 | 4.10 ± 0.08 | 4.00 ± 0.08 |
| 10            | 3.98 ± 0.01               | 5.42 ± 0.01              | 2.33 ± 0.02                       | 4.49 ± 0.04            | 0.34 ± 0.01                       | 0.49 ± 0.03             | 1.8 × 10³ ± 0.07 | 2.4 × 10¹ ± 0.03 | 4.07 ± 0.06 | 4.00 ± 0.09 |
| 20            | 4.25 ± 0.02               | 5.66 ± 0.01              | 2.67 ± 0.02                       | 4.63 ± 0.04            | 0.41 ± 0.01                       | 0.57 ± 0.02             | 2.5 × 10³ ± 0.07 | 2.9 × 10¹ ± 0.03 | 4.04 ± 0.08 | 3.99 ± 0.09 |
| 30            | 4.34 ± 0.03               | 5.83 ± 0.01              | 2.92 ± 0.05                       | 5.07 ± 0.04            | 0.43 ± 0.01                       | 0.62 ± 0.04             | 2.9 × 10³ ± 0.07 | 3.3 × 10¹ ± 0.03 | 4.02 ± 0.08 | 3.97 ± 0.07 |
| 40            | 4.69 ± 0.02               | 5.63 ± 0.01              | 3.18 ± 0.02                       | 5.42 ± 0.04            | 0.47 ± 0.06                       | 0.71 ± 0.04             | 3.2 × 10³ ± 0.07 | 4.9 × 10¹ ± 0.03 | 4.00 ± 0.06 | 3.96 ± 0.08 |
| 50            | 4.77 ± 0.04               | 5.83 ± 0.01              | 3.26 ± 0.03                       | 5.76 ± 0.04            | 0.49 ± 0.06                       | 0.74 ± 0.05             | 4.1 × 10³ ± 0.07 | 5.3 × 10¹ ± 0.03 | 3.98 ± 0.09 | 3.93 ± 0.07 |
| 60            | 4.89 ± 0.03               | 6.11 ± 0.01              | 3.43 ± 0.05                       | 6.38 ± 0.04            | 0.54 ± 0.06                       | 0.76 ± 0.02             | 5.5 × 10³ ± 0.07 | 6.9 × 10¹ ± 0.03 | 3.95 ± 0.08 | 3.91 ± 0.09 |
| 70            | 5.03 ± 0.03               | 6.27 ± 0.01              | 4.31 ± 0.04                       | 6.92 ± 0.05            | 0.59 ± 0.06                       | 0.81 ± 0.04             | 7.1 × 10³ ± 0.07 | 7.7 × 10¹ ± 0.03 | 3.92 ± 0.09 | 3.88 ± 0.05 |
| 80            | 5.14 ± 0.02               | 6.39 ± 0.01              | 4.94 ± 0.03                       | 7.34 ± 0.03            | 0.64 ± 0.06                       | 0.84 ± 0.02             | 9.7 × 10³ ± 0.07 | 9.8 × 10¹ ± 0.03 | 3.90 ± 0.09 | 3.85 ± 0.11 |
| 90            | 5.19 ± 0.02               | 6.48 ± 0.01              | 5.16 ± 0.03                       | 8.53 ± 0.03            | 0.68 ± 0.05                       | 0.89 ± 0.02             | 1.03 × 10² ± 0.05 | 1.07 × 10² ± 0.02 | 3.87 ± 0.07 | 3.82 ± 0.07 |

Note: n = 3. Results are expressed as mean values ± standard deviations. Values in bold are significantly different from other values in respective columns for different parameters (p < 0.05).

Abbreviations: OCFCs, optimized composite flour cookies; UWFCs, ungerminated wheat flour cookies.

4 CONCLUSION

The two-way approach of formulating a novel product for the preparation of cookies delivered a nutritious option in place of traditionally prepared cookies from ungerminated wheat. Various attempts have been made previously by researchers to successfully replace wheat flour with other legumes and cereals. Germinated cereal and legumes are able to replace the traditionally prepared wheat cookies for better digestibility and nutrition. Functional and pasting properties of legume-blended flour have shown the suitability of substitution and utilization of different flours from germinated grains. Owing to alteration in pasting properties and functional characteristics, cookies prepared from the composite flour exhibited soft texture cookies. Textural properties of OCFCs were softer with slightly dark color. All age groups prefer these kinds of cookies with soft texture and high digestibility. The shelf life of OCFCs was found quite comparable with that of UWFCs, whereas sensory score has shown the acceptability of product on a 5-point scale. Shelf life could be extended to a longer period by using specialized packaging techniques.

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CONFLICT OF INTEREST

The authors declare no conflict of interest with respect to this manuscript.

AUTHOR CONTRIBUTIONS

Charanjit Singh Riar conceived and supervised the concept, and Mandeep Singh Sibian performed the experiments, analyzed the data, and wrote the manuscript.

ETHICS STATEMENT

This article does not contain any human and animal subjects for experiment.

DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available from the corresponding author upon reasonable request.

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REFERENCES

Adebowale, A. A., Adegoke, M. T., Sanni, S. A., Adegunwa, M. O., & Fetuga, G. O. (2012). Functional properties and biscuit making potentials of sorghum-wheat flour composite. American Journal of Food Technology, 7(6), 372–379.
Mugil, D., Barak, S., & Khatak, B. S. (2017). Cookie texture, spread ratio and sensory acceptability of cookies as a function of soluble dietary fiber, baking time and different water levels. LWT-Food Science and Technology, 80, 537–542.

Myers, R. H., & Montgomery, D. C. (2002). Response surface methodology: Product and process optimization using designed experiments (2nd ed.). New York: John Wiley & Sons.

Naczk, M., Diosady, L. L., & Rubin, L. J. (1985). Functional properties of canola meals produced by a two phase solvent extraction method. Journal of Food Science, 50(6), 1685–1688.

Nagi, H. P. S., Kaur, J., Dar, B. N., & Sharma, S. (2012). Effect of storage period and packaging on the shelf life of cereal bran incorporated biscuits. American Journal of Food Technology, 7(5), 301–310.

Nkhata, G. S., Ayua, E., Kamau, H. E., & Shingiro, J. (2018). Fermentation and germination improve nutritional value of cereals and legumes through activation of endogenous enzymes. Food Science and Nutrition, 6(8), 2446–2458. https://doi.org/10.1002/fsn3.846

Noor Aziah, A. A., Mohamad Noor, A. Y., & Ho, L. H. (2012). Physicochemical and organoleptic properties of cookies incorporated with legume flour. International Food Research Journal, 19(4), 1539–1543.

Noorfarahzilah, M., Lee, J. S., Sharifudin, M. S., Mohd Fadzelly, A. B., & Hasmadi, M. (2014). Applications of composite flour in development of food products. International Food Research Journal, 21(6), 2061–2074.

Oupadissakoon, C., & Young, C. T. (1984). Modeling of roasted peanut flavor for some Virginia-type peanuts from amino acid and sugar contents. Journal of Food Science, 49(1), 52–58.

Ribotta, P. D., Armulphi, S. A., León, A. E., & Anón, M. C. (2005). Effect of soybean addition on the rheological properties and bread making quality of wheat flour. Journal of the Science of Food and Agriculture, 85(11), 1889–1896.

Sanchez, H. D., Osella, C. A., & Torre, M. A. (2004). Use of response surface methodology to optimize gluten-free bread fortified with soy flour and dry milk. Food Science and Technology International, 10(1), 5–9.

Sibian, M. S., Saxena, D. C., & Riar, C. S. (2016). Effect of pre and post germination parameters on the chemical characteristics of Bengal gram (Cicer arietinum). LWT-Food Science and Technology, 65, 783–790. https://doi.org/10.1016/j.lwt.2015.09.012

Sibian, M. S., Saxena, D. C., & Riar, C. S. (2017). Effect of germination on chemical, functional and nutritional characteristics of wheat, brown rice and triticale: A comparative study. Journal of Science of Food and Agriculture, 97(13), 4643–4651. https://doi.org/10.1002/jsfa.8336

Sindhuja, A., Sudha, M. L., & Rahim, A. (2005). Effect of incorporation of amaranth flour on the quality of cookies. European Food Research Technology, 221(5), 597–601. https://doi.org/10.1007/s00217-005-0039-5

Singh, R., Singh, G., & Chauhan, G. S. (2000). Development of soy-fortified biscuits and shelf-life studies. Journal of Food Science and Technology, 37(3), 300–303.

Stabnikova, O., Antoniuk, M., Stabnikov, V., & Arsen’eva, L. (2019). Ukrainian dietary bread with selenium-enriched soya malt. Plant Foods for Human Nutrition, 74(2), 157–163. https://doi.org/10.1007/s11130-019-00731-z

Yadav, D. N., Sharma, M., Chikara, N., Anand, T., & Bansal, S. (2014). Quality characteristics of vegetable-blended wheat-pearl millet composite pasta. Agricultural Research, 3(3), 263–270. https://doi.org/10.1007/s40003-014-0117-7

Yamazaki, W. T. (1953). An alkaline water retention capacity test for the evaluation of cookie baking potentialities of soft winter wheat flours. Cereal Chemistry, 30, 242–246.

Yusufu, P. A., Netala, J., & Opega, J. L. (2016). Chemical, sensory and microbiological properties of cookies produced from maize, African yam bean and plantain composite flour. Indian Journal of Nutrition, 3(1), 1–5.

Zoulias, E. I., Piknis, S., & Oreopoulou, V. (2000). Effect of sugar replacement by polyols and acesulfame-K on properties of low fat cookies. Journal of the Science of Food and Agriculture, 80(14), 2049–2056.

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