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Process optimisation of functional cookies using multi-grain mix and casein hydrolysate#

S. Anishadas[1], R. L.Beena[2], S.N.Rajakumar[1], G.R. Jayadevan[3], B. Indu[1] and T. J. Bincy[1]

[1]Verghese Kurien Institute of Dairy and Food Technology, Thrissur-680651, Kerala, India, [2]College of Dairy Science and Technology, Kolahalamedu, Idukki-685501, Kerala, India, [3]College of Dairy Science and Technology, Pookode, Wayanad-673576, Kerala, India, Kerala Veterinary and Animal Sciences University Kerala, India

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Abstract

Today's busy lifestyle has made women more susceptible to imbalances in nutrient intake. Calcium deficiency leading to osteoporosis at ageold has become a common threat. The present study was a continuation of an ongoing work done for the optimisation of a functional composite flour premix proven to have good calcium binding properties. Ready-to-eat snacks are liked by all and is a promising path for neutraceutical supplementation. In the current study, the optimised premix was used to make functional cookies. Casein hydrolysate, a bioactive compound with various health benefits, was used as the functional ingredient at 7.6% degree of hydrolysis. A nine-grain flour mix was used as the base, which was optimised based on the Recommended Dietary Allowance (RDA) for Indian women as proposed by the Indian Council of Medical Research (ICMR) in 2020. Response Surface Methodology (RSM) was employed for the optimisation of functional cookie using three independent factors viz levels of addition of casein hydrolysate, baking temperature and baking time. The various sensorial aspects of the developed product were evaluated and the results were statistically analysed using SPSS 22 software. The optimal conditions for achieving maximum desirability were found to be 14.62% addition of casein hydrolysate and a temperature-time combination of 170.5 °C and 25.96 minutes. Product characterisation was done with respect to its proximate composition. The moisture, crude protein, crude fat, ash, crude fibre, carbohydrate, calcium and energy values were found to be 4.46%, 16.18%, 21.68%, 3.27%, 3.8%, 55.05%, 1.3% and 480.12 Kcal/100g, respectively.

Keywords : *Functional cookies, composite flour, degree of hydrolysis*

In recent years, food habits of consumers have shifted due to urbanisation and changing lifestyles, increasing the preference for ready-to-eat (RTE) food products. These products require no cooking and have become more popular due to its convenience. The most popular RTE food products are bakery products, dried and fried foods, canned items, sweet and savoury snacks. These light weight, shelf-stable, and nutritionally enhanced options appeal to consumers seeking quick meals (Temgire *et al*., 2021). Cookies, a favourite bakery product, are popular among all age groups due to its convenience and ready-to-eat nature. The major ingredient in traditional cookies is refined wheat flour which is high in starch, sugar, fat and low in protein and crude fibre. This causes various life style diseases like diabetes, obesity, cancer, constipation, high blood cholesterol and coronary heart disease. Hence there is a need to improve the nutritive quality of cookies for today's health conscious consumers (Awolu *et al*., 2017). In order to address the growing expectations of a

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**Corresponding author: beenarl@kvasu.ac.in, Ph. 9947367580*

healthy and balanced diet, the food sector is exploring new functional food products.

A natural or processed food is considered functional if it contains active ingredients in specified quantitative and qualitative levels and has been shown to have measurable health benefits (Vieira *et al*., 2020). Singh and Singh (2021) optimised composite flour cookies prepared from germinated triticale, kidney bean, and chickpea and found that the protein content and crude fiber was increased than that of the wheat cookies. The protein and crude fiber content of composite flour cookies were found to be 11.81 ± 0.05 % and 5.79 ± 0.02 % and that of wheat cookies were reported to be 7.84 ± 0.02 % and 1.93 ± 0.03 %, respectively. Researchers are paying close attention to the growing development of food products made using composite flour, particularly those used in baked food products (Noorfarahzilah *et al*., 2014). Aljobair (2022) prepared cookies using sorghum and millet composite flour that are rich in nutrients and bioactive compounds, which can enhance nutrition and health. There are many factors present in cereals and pulses, such as phytates and oxalates that hinders the absorption of minerals especially calcium in the body. It has been shown that they chelate metal ions like calcium, magnesium, zinc, copper, iron, and molybdenum to create insoluble complexes that are difficult for the digestive system to absorb, impairing its role in bone health, enzyme function, nerve transmission, and blood clotting. This low calcium absorption causes teeth and bone deterioration as well as problems with the blood clotting mechanism (Bhandari and Kawabata, 2006). The 2022 annual report of the International Osteoporosis Foundation estimates that one in three women and one in five men experience an osteoporosis-related fracture at age-old. Casein hydrolysate has the ability to solubilize divalent minerals like Ca^{2+},thereby improving its absorption into the body (Bennett *et al*., 2000). In addition to its calcium absorption ability, it has many other functional properties such as ACE-inhibitory activity, improved digestibility, reduced allergenicity, antimicrobial properties, antioxidant activities, antithrombotic effects, cholesterol lowering effects, immunomodulatory effects and opioid effects (Shaik, 2021). Beena (2022) reported the high calcium absorption property of casein hydrolysate in an *in vivo* study using a composite flour premix with added casein hydrolysate. This premix contained a cereal blend (wheat, barley, foxtail millet, finger millet), pulse blend (green gram, horse gram, green peas, soybean) and an oil seed (black sesame) at an ingredient composition of 62.25%, 33.99 % and 3.76 % oilseed respectively of the total mix. It was optimised based on the daily nutrient requirement for the Indian women proposed by the Indian Council of Medical Research (ICMR) in 2020. Due to the high market demand in ready-to-eat snacks, an attempt is made to standardise the production of functional cookies using the optimised composite flour premix. The consideration made is the production of a highly acceptable product with minimum possible heating intensity to protect the functional parameters, which need further validation.

Materials and methods

The raw materials required for the preparation of cookies were purchased from the local market.

Composite flour

All the grains were thoroughly cleaned, dried and sauted in low flame for 5 minutes. They were then ground separately, sieved and stored in separate air tight containers till further use. Composite flour was prepared by mixing the correct proportions of each type of flour, as suggested by Beena *et al.* (2022).

Casein hydrolysate

Food-grade casein was hydrolysed using food-grade alcalase enzyme according to the method done by Beena (2022) as described here. 15 g of casein was mixed with 85 ml distilled water to prepare the casein solution, which was heated to 60 °C for complete dissolution. It was heated to 85 °C for 15 minutes to inactivate the native proteases and the pH was adjusted to 8.5 using 2 N food-grade sodium hydroxide solution. Alcalase was added @ 12 DU/g protein and the hydrolysis was done at 60 °C, maintaining the pH continuously with 1 N food-grade sodium hydroxide solution till 7.6% degree of hydrolysis. The enzyme was inactivated by heating the mixture to 85 °C for 15 minutes. The unhydrolyzed casein was precipitated out by lowering the pH to 4.6 with 2 N HCl and removed by centrifuging at 6000 x g for 20 minutes at 4 °C. The soluble casein hydrolysate was collected, freeze-dried, and stored at -20 °C till further use.

Functional Cookies

Cookies were prepared according to the procedure done by Patil *et al*. (2018) with slight modifications as below.The ingredients were taken as given in Table 1. The dry ingredients were sieved, and the

Table 1. Ingredients for functional cookie

Ingredients	Quantity(%)
Composite flour	50
Casein hydrolysate	7.313
Milk powder	8.33
Sugar	20.83
Butter	25
Baking soda	0.208
Baking powder	0.208
Dry chilly	0.833
Green chilly	0.833
Ginger	1.666
Fennels	0.833
Shallots	12.5
Curry leaves	1.666

masala mix was prepared by crushing dried chillies, green chillies, ginger, fennel, shallots, and curry leaves in a mortar and pestle. Butter at room temperature was blended well with powdered sugar and all the dry ingredients. After that, the masala mix was added and thoroughly mixed to form a smooth dough. The dough was apportioned into 15 g balls and moulded before being placed in a preheated oven at 170°C. Baking was done for 25 minutes, after which the balls were cooled to room temperature and packed in airtight containers.

Blending butter with sugar and milk powder
↓
Addition of all dry ingredients (including casein hydrolysate) and masala mix
↓
Mixing & kneading to form a smooth dough
↓
Moulding
↓
Baking at 170˚C/ 25 min
↓
Cooling (at room temperature)
↓
Packaging & storage

Fig.1. Flow chart for functional cookie preparation

Sensory evaluation

Samples for sensory analysis were coded with three-digit random numbers. A hedonic rating scale (9-point scale; 1 = dislike extremely, 9 = like extremely) was used to score flavour, colour and appearance, body and texture and overall acceptability. Seven semi-trained judges participated in the analysis, which was conducted with four replications and statistically analysed*.*

Proximate analysis of the optimised cookie

The moisture, protein, fat, ash, calcium, carbohydrate, crude fiber and energy value of the functional cookies were analysed as per the standard procedure by the Association of Official Analytical Collaboration (AOAC), International 2016**.**

Optimisation of the process parameters

Preliminary trials were conducted to determine the minimum and maximum levels of independent factors (quantity of casein hydrolysate addition, baking time and temperature) for product preparation based on sensory evaluation. The proportion of the functional ingredient was determined at its maximum threshold level and the other independent factors at their minimum level possible, to develop functional cookies with minimum heat impact. RSM models were used to optimise the relationship among these three independent variables. The software used for the study of the interaction level was Design Expert 9.0 version of Stat-Ease, Inc, 2021E, Hennepin Avenue, Minneapolis, USA. The CCRD of these three independent factors suggested 20 trials. Cookies were made using the various combinations and given for sensory evaluation. The responses were fed to the software to optimise the level of the factors studied. The minimum and maximum levels of the factors and the design matrixes are depicted in Tables 2 and 3 respectively. The basic model equation to fit the data is given by the equation:

$$Y = \beta_0 + \sum_{i=0}^{n} \beta_i X_i + \sum_{i=0}^{n} \beta_{ii} X_i^2 + \sum_{i \neq i=1}^{n} \beta_{ij} X_i X_j$$

Where Y is the predicted response, β_0 is the intercept, n is the number of variables studied, β_i , β_{ii} and β_{ij} are the linear (effect of that single factor), quadrative (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.

Using Central Composite Rotatable Design (CCRD), 20 experiments were conducted to evaluate the effects of casein hydrolysate addition, temperature, and time. The constraints and criteria for optimisation is depicted in Table 4.

The estimated optimal response values using the desirability function is shown in the table 5. The fitted models predicted coefficients of 7.639 for flavour, 7.598 for colour, 7.557 for body and texture and 7.549 for overall acceptability, with these values corresponding to an optimised combination of 14.626% casein hydrolysate, a temperature of 170.522°C and a baking time of 25.964 minutes. Sensory evaluation of the product prepared using these optimum parameters was carried out using a 9-point

Table 2. Levels of independent variables for experimental design

Independent variable	minimum	maximum
A: Levels of addition of casein hydrolysate (%)	14	16
B: Temperature (˚C)	160	175
C: Time (minutes)	20	30

Table 3. Design matrix used for the optimisation of the functional cookies

	FACTORS		
Standard order	A: Levels of addition of casein hydrolysate (%)	B: Temperature (°C)	C: Time (minutes)
1	14	160	20
2	16	160	20
3	14	175	20
4	16	175	20
5	14	160	30
6	16	160	30
7	14	175	30
8	16	175	30
9	13.3182	167.5	25
10	16.6818	167.5	25
11	15	154.887	25
12	15	180.113	25
13	15	167.5	16.591
14	15	167.5	33.409
15	15	167.5	25
16	15	167.5	25
17	15	167.5	25
18	15	167.5	25
19	15	167.5	25
20	15	167.5	25

Table 4. Constraints and criteria for optimisation of functional cookies with different levels of addition of casein hydrolysate, temperature and time

Name	Goal	Lower Limit	Upper Limit
A:Casein hydrolysate (%)	is in range	14	16
B:Temperature (˚C)	is in range	160	175
C:Time (minutes)	is in range	20	30
Color	maximize	5.3	7.62
Flavour	maximize	5.5	7.75
Body & Texture	maximize	5.3	7.6
Overall Acceptability	maximize	5.35	7.68

Table 5. Solution obtained after response surface analysis

Casein hydrolysate (%)	14.626
Temperature (˚C)	170.522
Time (minutes)	25.964
Color	7.598
Flavour	7.639
Body and texture	7.557
Overall acceptability	7.549
Desirability	0.966

hedonic scale. The sensory panelists were served two optimised cookies prepared in the same combinations as mentioned above with different labelling. The experiment was repeated four times to validate the predicted values, and Table 8 presents the mean observed sensory scores along with their t-values.

Results and discussion

The impact of three independent variables (levels of addition of casein hydrolysate addition, temperature and time of baking) on the sensory scores of functional cookies is depicted in Table 6 and 7.

Effect on flavour

The sensory scores for the flavour of the functional cookies, ranged from 5.5 to 7.75. The quadratic equation derived from the Response Surface Analysis (RSA) captures the effects of addition of levels of casein hydrolysate(A), temperature(B) and time(C).

$$Flavour = 7.47387 - 0.318386 * A + 0.196503 * B + 0.000133319 * C - 0.26625 * AB - 0.34125 * AC + 0.34625 * BC - 0.262185 * A^2 - 0.456639 * B^2 - 0.385928 * C^2$$

The maximum score was observed for standard order 7 (casein hydrolysate: 14%, temperature: 175 °C, baking time: 30 minutes), while the least score was observed for standard order 6 (casein hydrolysate: 16%, temperature: 160 °C, baking time: 30 minutes), with scores of 7.75 and 5.5, respectively. The model demonstrated a significant F-value of 68.33, with a non-significant lack of fit, indicating the model's accuracy. The coefficient of determination (R^2) was 98.4%, reflecting that 98.4% of

Table 6. Sensory characteristics of functional cookies with different levels of addition of casein hydrolysate, temperature and time

Standard order	Response 1	Response 2	Response 3	Response 4
	Flavour	Colour	Body and texture	Overall acceptability
1	6.2	5.6	5.3	6.4
2	6.8	5.42	6.2	5.35
3	6.3	6.35	6.1	6.51
4	5.9	6.4	5.9	6.3
5	6.2	6.32	6.1	6.1
6	5.5	5.8	6.1	5.67
7	7.75	6.4	7.6	6.32
8	5.92	6.2	6.5	6.6
9	7.4	7	6.5	7.1
10	6.2	6.5	6.4	6.32
11	5.8	6	6.2	5.93
12	6.7	7.1	7.2	6.72
13	6.5	5.3	5.32	5.4
14	6.4	5.8	6.5	5.9
15	7.5	7.62	7.4	7.48
16	7.5	7.6	7.4	7.58
17	7.6	7.52	7.4	7.53
18	7.42	7.6	7.42	7.68
19	7.45	7.46	7.4	7.5
20	7.35	7.52	7.32	7.4

the variation in flavour response was accounted for by the variables. An adequate precision ratio of 27.2601 confirmed a strong signal. The F-value exceeded the critical value at the 5% significance level ($p < 0.05$), affirming the model's suitability for predicting flavor outcomes. The p-values indicated that the levels of addition of casein hydrolysate, temperature and time significantly impacted the flavour scores of the functional cookies. Among the three factors, the addition of levels of casein hydrolysate had a negative influence on flavour scores at quadratic levels, while the other two factors had a positive influence. Shaik (2021) reported a similar negative effect on addition of levels of casein hydolysate-zn solution in biscuit and cup cake prepared from refined wheat flour.

Effect on colour and appearance

The sensory scores for the colour of the functional cookies, ranged from 5.3 to 7.62, were modeled using a quadratic equation derived from the Response Surface Analysis (RSA). This equation captures the effects of addition of levels of casein hydrolysate(A), temperature(B), and time(C).

$$\text{Colour} = 7.55802 - 0.123813 * A + 0.297285 * B + 0.131135 * C + 0.06875 * AB - 0.07375 * AC - 0.15625 * BC - 0.314629 * A^2 - 0.38534 * B^2 - 0.738893 * C^2$$

The maximum score was observed for standard order 15 (casein hydrolysate: 15%, temperature: 167.5 °C, baking time: 25 minutes), while the least score was observed for standard order 13 (casein hydrolysate: 15%, temperature: 167.5 °C, baking time: 16.591 minutes), with scores of 7.62 and 5.3, respectively. The model demonstrated a significant F-value of 125.22 , with a non-significant lack of fit, indicating the model's accuracy. The coefficient of determination (R^2) was 99.12 %, reflecting that the variables accounted for 99.12% of the variation in colour and appearance response. An adequate precision ratio of 31.8265 confirmed a strong signal. The F-value exceeded the critical value at the 5% significance level ($p < 0.05$), affirming the model's suitability for predicting colour outcomes. The p-values indicated that the levels of addition of casein hydrolysate, temperature and time significantly impacted the colour scores of the functional cookies. Among the three factors, the addition of levels of casein hydrolysate had a negative influence on colour scores at quadratic levels, while the other two factors had a positive influence. Galla *et al.* (2017) reported a similar negative effect on color as the percentage of spinach powder added to cookies increased.

Effect on Body and Texture

The sensory scores for the body and texture of the functional cookies, ranged from 5.3 to 7.6, were modeled using a quadratic equation derived from the Response Surface Analysis (RSA). This equation captures the effects of levels of addition of casein hydrolysate(A), temperature(B), and time(C).

$$\text{Body and texture} = 7.39211 - 0.041604 * A + 0.298882 * B + 0.350338 * C - 0.275 * AB - 0.225 * AC + 0.175 * BC - 0.346151 * A^2 - 0.257763 * B^2 - 0.53707 * C^2$$

Table 7. Estimated parameters of model for sensory attributes and responses of functional cookies for women with different levels of addition of casein hydrolysate, temperature and time

Partial Coefficients	Sensory characteristics			
	Flavour	Colour and appearance	Body and texture	Overall acceptability
Intercept	7.473	7.558	7.392	7.528
Linear				
A-CH	-0.318**	-0.123**	-0.041**	-0.199**
B-Temperature	0.1965**	0.297**	0.298**	0.259**
C-Time	0.000131ns	0.131**	0.350**	0.0710*
Interaction				
AB	-0.266**	0.068ns	-0.275**	0.193**
AC	-0.341**	-0.073ns	-0.225**	0.138**
BC	0.346**	-0.156**	0.175**	0.0112ns
Quadratic				
A^2	-0.262**	-0.314**	-0.346**	-0.287**
B^2	-0.456**	-0.385**	-0.257**	-0.424**
C^2	-0.385**	-0.738**	-0.537**	-0.662**
Model Fit Statistics				
Lack of fit	3.323ns	4.341ns	2.430ns	1.684ns
Model F value	68.326**	125.221**	524.751**	97.869**
R^2	0.983	0.991	0.997	0.988
Press	0.961	0.677	0.122	0.647
Adeq precision	27.26	31.826	71.545	28.543

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

Table 8. Verification of the predicted values of the experimental responses

Attributes	Predicted value	Observed value	t-value	p-value
Flavour	7.63±0.13	7.75±0.250	0.444ns	0.1067
Colour	7.59±0.11	7.5±0.289	0.339ns	0.0665
Body and texture	7.55±0.04	7.5±0.289	0.197ns	0.176
Overall acceptability	7.54±0.11	7.5±0.289	0.170ns	0.2905

All observations are mean values of four replications with SE, ns- non-significant (p ≥ 0.05)

The maximum score was observed for standard order 7 (casein hydrolysate: 14%, temperature: 175 °C, baking time: 30 minutes), while the least score was observed for standard order 1 (casein hydrolysate: 14%, temperature: 160 °C, baking time: 20 minutes), with scores of 7.6 and 5.3, respectively. The model demonstrated a significant F-value of 524.75, with a non-significant lack of fit, indicating the model's accuracy. The coefficient of determination (R^2) was 99.79%, reflecting that the variables accounted for 99.79% of the variation in body and texture response. An adequate precision ratio of 71.5453 confirmed a strong signal. The F-value exceeded the critical value at the 5% significance level ($p < 0.05$), affirming the model's suitability for predicting body and texture outcomes. The p-values indicated that the levels of addition of casein hydrolysate, temperature and time significantly impacted the body and texture scores of the functional cookies. Among the three factors, the addition of levels of casein hydrolysate had a negative influence on body and texture scores at quadratic levels, while the other two factors had a positive influence. Tyagi *et al.* (2020) reported a similar negative influence on body and texture of cookies optimised using *Tinospora cordifolia* (TC) powder. The study found that sensory scores for body and texture decreased as the concentration of TC powder increased from 0 to 12%.

Effect on overall Acceptability

The sensory scores for the overall acceptability of the functional cookies, ranged from 5.35 to 7.68, were modeled using a quadratic equation derived from the Response Surface Analysis (RSA). This equation captures the effects of addition of levels of casein hydrolysate(A), temperature(B), and time(C).

$$\text{Overall acceptability} = 7.52811-0.199299 * A + 0.259109 * B + 0.0710922 * C + 0.19375 * AB + 0.13875 * AC + 0.01125 * BC -0.287895 * A^2-0.424013 * B^2-0.662661 * C^2$$

The maximum score was observed for standard order 18 (casein hydrolysate: 15%, temperature: 167.5 °C, baking time: 25 minutes), while the least score was observed for standard order 2 (casein hydrolysate: 16%, temperature: 160 °C, baking time: 20 minutes), with scores of 7.68 and 5.35, respectively. The model demonstrated a significant F-value of 97.87, with a non-significant lack of fit, indicating the model's accuracy. The coefficient of determination (R^2) was 98.88%, reflecting that the variables accounted for 98.88% of the variation in overall acceptability response. An adequate precision ratio of 28.5430 confirmed a strong signal. The F-value exceeded the critical value at the 5% significance level ($p < 0.05$), affirming the model's suitability for predicting overall acceptability outcomes. The p-values indicated that the levels of addition of casein hydrolysate, temperature and time significantly impacted the overall acceptability scores of the functional cookies. Among the three factors, the addition of levels of casein hydrolysate had a negative influence on overall acceptability scores at quadratic levels, while the other two factors had a positive influence. Aziza (2019) found that biscuits made with a 10% CNH-Fe complex solution achieved a score of 8.58, indicating a favourable level of acceptance from the judging panel. However, increasing the concentration of the CNH-Fe solution further negatively impacted the overall acceptability score of the cookies.

Statistical verification of the predicted Value

The results showed no significant difference between the predicted and observed values, confirming the accuracy of the casein hydrolysate levels, temperature and processing time used in the study. The response surface plots showing the effects of levels of casein hydrolysate addition, temperature, and time on flavor, color, body and texture and overall acceptability are depicted below (Fig. 2).

Proximate analysis

The functional cookies optimised based on sensory responses were then evaluated for their nutritive value. The results of the proximate analysis are depicted in Table 9. In comparison, Shaik (2021) reported a moisture content of 3.35% for the control wheat biscuit and 4.23% for the casein hydrolysate-zinc complex incorporated wheat biscuit. This indicates that the optimised cookies have a higher moisture content than both the control and the casein hydrolysate-zinc complex biscuits. Shaik (2021) reported protein, fat, ash and carbohydrate contents of 7.58%, 21.53%, 0.97% and 65.68%, respectively, in CNH-Zn incorporated biscuits. In contrast, the optimised cookies exhibited a higher protein content of 16.18%, indicating a significant enhancement in their nutritional profile. This increase in protein may provide greater health benefits and improved functional properties. The fat content in both formulations is comparable, with CNH-Zn incorporated biscuits containing 21.53% and the optimised cookies showing 21.68%. The ash content of the optimised cookies is also notably higher at 3.27%, compared to 0.97% in the CNH-Zn incorporated biscuits. This increase may indicate a higher mineral content, which could enhance the overall nutritional value of the cookies. Conversely, the carbohydrate content in the optimised cookies is lower at 55.05%, compared to 65.68% in the CNH-Zn incorporated biscuits. This reduction may affect the energy density and textural properties of the cookies, potentially making them a more balanced option for consumers seeking lower carbohydrate intake. In summary, the optimised cookies demonstrate improved protein and ash content while maintaining similar fat levels and exhibiting lower carbohydrate content compared to the CNH-Zn incorporated wheat biscuits. These findings suggest that the optimised cookies may offer enhanced nutritional benefits. The crude fibre content of biscuits produced from a blend of wheat and velvet bean flour varied significantly ($p < 0.05$), ranging from 1.03% to 1.40% was reported by Ezegbe *et al.* (2023). This content increased with the addition of velvet bean flour. In comparison, the optimised cookies in this study exhibited a higher crude fibre content of 3.8%. This indicates that the optimised cookies provide a more substantial source of dietary fibre, which is essential for maintaining intestinal health and promoting digestive transit. Overall, the increased fibre content in the optimised cookies may offer enhanced health benefits compared to the biscuits formulated with velvet bean flour. Igbabul (2018) reported the calcium content of wheat

Fig.3. Functional cookies for women

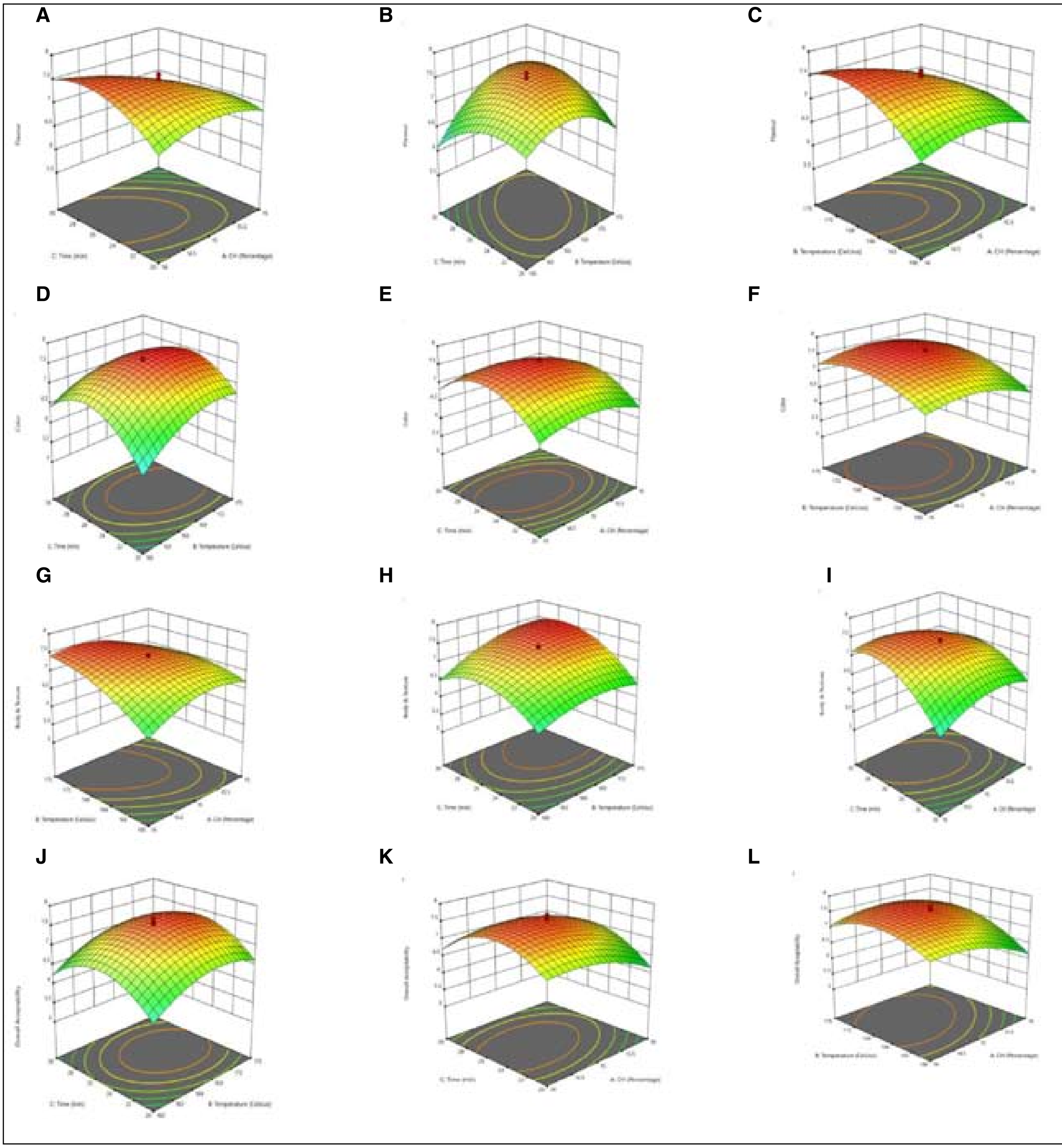

Fig. 2. Response surface plots of the sensory responses as affected by the independent variables

A: Effect of time and casein hydrolysate percentage on flavour B: Effect of time and temperature on flavor C: Effect of temperature and casein hydrolysate percentage on flavor D: Effect of time and temperature on colour E : Effect of time and Casein Hydrolysate % on colour F: Effect of temperature and casein hydrolysate % on colour. G: Effect of temperature and casein hydrolysate % on body and texture H: Effect of time and temperature on body and texture I: Effect of time and casein hydrolysate % on body and texture J: Effect of time and temperature on overall acceptability K: Effect of time and casein hydrolysate % in overall acceptability L: Effect of temperature and Casein Hydrolysate % on overall acceptability.

cookies as 0.02126%. In contrast, the optimised cookies in this study demonstrated a significantly higher calcium content of 1.3%. This substantial increase indicates that the optimised cookies may offer enhanced nutritional benefits, particularly in terms of calcium intake, which is essential for bone health and overall metabolic function. The higher calcium content in the optimised cookies suggests they could serve as a more effective dietary source of this important mineral compared to traditional wheat cookies. Mounika *et al.* (2017) reported an energy content of 521 kcal/100g for their cookies, which is higher than the 480.12 kcal/100g observed in the optimised cookies developed in this study. This reduction in energy content may be attributed to the formulation adjustments

Table 9. Proximate Analysis

Particulars	Composition
Moisture (%)	4.46±0.033
Crude protein (dry matter basis) (%)	16.18±0.005
Crude fat (dry matter basis) (%)	21.68±0.03
Crude fibre (%)	3.8±0.057
Ash (%)	3.27±0.013
Calcium (%)	1.317±0.044
Carbohydrate (dry matter basis) (%)	55.05±0.07
Energy value (Kcal/100g)	480.12 ± 0.12

made to enhance the nutritional profile of the optimised cookies.

Conclusion

In the current work, the production process of functional cookies for women using a composite flour containing nine grains was optimised. The results of the proximate analysis indicated a high nutritive value, making them a valuable option to be included in women's diet. The high protein content shows that the product can be included in the high-protein segment foods. As the wheat flour is substituted by a healthy combination of various cereals and pulses, the final product will get a pool of essential amino acids. Moreover the gluten allergenicity can be minimised. The base flour-mix had proved good calcium binding property in the earlier study and the property is believed to have extended in the newly developed product. Care was taken to minimise the temperature and time of heat treatment for maximum retention of the functional ingredient in the product. Further research is warranted to validate the calcium absorption property of the developed cookie. It will definitely benefit the women community in the society.

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Conflicts of interest

The authors declare that they have no conflict of interest

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