Journal of Food Engineering and Technology 2025;14(1):1-11
https://doi.org/10.32732/jfet.2025.14.1.1

Effect of High Protein Formulation on Rheological, Sensory and Microstructure of Cookies

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Received: 7 November 2024; Accepted: 8 December 2024; Available online: 10 January 2025

Abstract: This study investigates the impact of high-protein formulations on the rheological, sensory, and microstructural properties of cookies, with an emphasis on using plant- and dairy-based protein sources. Protein plays an essential role in the diet, providing amino acids necessary for muscle, healthy bone, tissue repair, growth in children, adolescents, and pregnant women. Despite the known benefits of plant-based proteins, limited research has focused on their effect in high-protein bakery products, particularly cookies. In this study, three cookie formulations were developed using pea protein isolate, soy protein isolate, and a combination of whey protein isolate with skim milk powder, along with almonds for enhanced nutritional value. Control cookies were prepared with wheat flour. Proximate analysis showed a significant increase in protein content with 53.22% in the pea protein isolate cookies, 46.76% in the soy protein isolate cookies, and 49.92% in the whey and skim milk powder cookies in comparison with control which was 12%. Farinograph analysis indicated increased breakdown times for the experimental doughs, ranging from 13.2 to 19.1 minutes compared to 9 minutes for the control, suggesting improved dough stability. Microstructural analysis revealed a porous structure in the cookies, likely resulting from trapped air during baking, which enhances texture and lightness. Observations showed that interactions between protein isolates and starch granules create structural diversity, contributing to the cookie's crispness and chewiness. Sensory evaluation favored the whey protein isolate and skim milk powder formulation, which scored the highest in appearance, flavor, and texture attributes. Overall acceptability ratings were favorable across all samples, with the cookie containing whey protein + Skim milk powder receiving the highest score (8.5) among the high-protein cookies. This study demonstrates the potential of high-protein cookie formulations as nutritionally valuable food products. Further research is recommended to examine the effects of varying protein proportions on the sensory and rheological properties of these formulations.
Keywords: Cookies; Rheology; Proximate composition; Micro structure; X-ray diffraction.

1. Introduction

Cookies are a popular dessert choice worldwide, offering a variety of flavors, textures, and shapes to suit diverse preferences. The global cookie market was valued at USD 30.62 billion in 2018, with an expected compound annual growth rate of 5.3% between 2019 and 2025, reflecting the sustained demand for these products [1]. Despite this demand, cookies are often high in calories due to their high fat and sugar content, and their primary ingredient, wheat flour, which lacks essential nutrients and has a low nutrient density [2]. To address these concerns, some studies have explored alternative ingredients to improve the nutritional profile of cookies. For example, cookies made with whey and sucralose exhibited 10.5% protein, while red kidney bean protein isolate raised the protein content by 115% compared to control, although higher supplementation levels negatively impacted texture and color [3, 4]. In another study, combining whey protein (WP) and soy protein isolate (ISP) improved overall acceptance in consumer tests, particularly when used in a 1:1 ratio [5].

Proteins play a fundamental role in building and repairing tissues, providing structural support, and enhancing immune function through the production of antibodies. They are also crucial for muscle contraction, enabling physical activity and movement [6]. Protein deficiencies can lead to serious health issues, particularly in young children, where insufficient intake is associated with conditions like kwashiorkor, marasmus, impaired growth, cognitive issues, and anemia [7, 8]. The FDA recommends a daily protein intake of 50 grams per individual to meet these essential needs [7]. For pregnant women, adequate protein intake is especially critical to support fetal development, tissue formation, and organ growth. Insufficient protein during pregnancy can result in complications such as low birth weight and developmental delays in the baby, highlighting the importance of a protein-rich diet for both maternal and fetal health [9].

The increasing awareness of the link between nutrition and health has driven demand for convenient, health-focused food options. Protein-fortified snacks like cookies could serve both nutritional and functional roles, including as emergency foods in times of war or natural disasters [4]. In this study, in response to the importance of protein and the widespread popularity of snack foods, three high-protein cookie formulations were developed using pea protein isolate (PPI), soy protein isolate (SPI), and a combination of whey protein isolate (WPI) with skim milk powder (SMP). The findings of this study aim to contribute to the development of protein-enriched foods with potential health benefits.

2. Materials and Methods

2.1 Raw Materials

The ingredients utilized in this study comprised whole wheat flour, skim milk powder (SMP), sugar, palmolein oil as the fat source and almonds. All ingredients were procured from the local market. Whey protein isolate (WPI) was sourced from Polmlek sp, Poland, while pea protein isolate was obtained from Yantai Shuangta Food Co., Ltd. in Shandong province, China. Additionally, soy protein isolate (SPI) was supplied by Shiv Health Foods LLP in Kota.

2.2 Cookie Formulations

Four formulations of cookies were made as follows based on isolates of Pea, Soy and whey proteins including control with whole wheat flour (Atta) which is provided in Table 1.

Table 1: Composite flour mix - High Protein Cookies

Ingredients in Kgs	BCFM 11 (CONTROL)	BCFM 12	BCFM 13	BCFM 14
Whole Wheat flour	100	35.12	49.82	46.24
Pea isolate	-	58.06	-	
Soy isolate	-	-	43.4	
Skim Milk Powder	-	-	-	17.92
Whey protein isolate	-	-	-	29.03
Almond bits		6.8	6.8	6.8
Total	100	100	100	100

2.3 Proximate Analysis

Proximate analysis was conducted for the composite flour samples following standard procedures. The moisture, Ash, Fat, Protein contents was assessed according to AOAC Methods **(925.09; 942.05**; **920.39**; **981.10)** Carbohydrates was calculated by difference using the formula: Carbohydrates = 100% - (Moisture + Ash + Fat + Protein). Dietary fiber content was determined (**AOAC Method 991.43)** utilizing the enzymatic-gravimetric method. All analyses were performed in triplicate to ensure accuracy and reliability of the results.

2.4 Rheological properties

Farinograph studies were carried out according to standard AOAC (2023) [10] methods. The rheological parameters of all Cookie formulations and Control were assessed utilizing the Brabender farinograph-E (Brabender OHG, Duisburg, Germany).

2.5 Cookies preparation ingredients

The below ingredients were added to make protein rich cookies along with control (Table 2). This combination resulted in 20 to 24% protein in the final cookie product. Cookies made as per the process flow mentioned in the Figure 1. Dough was prepared using all four composite flour combinations for cookie production, as shown in Figure 2.

Before baking, the dough was rolled into sheets to achieve the desired thickness, as illustrated in the second stage of the figure. After the baking process, the cookies were formed and are depicted in the final stage of Figure 2.

2.6 Scanning electron microscopic (SEM) studies

The structural morphology of the cookies was investigated using a scanning electron microscope (Hitachi S 3400 N, Japan) at the Research Lab, University with Potential for Excellence (UPE), University of Mysore, Karnataka, India. The microstructure of the samples was analyzed at five different magnifications, with a power level set at approximately 5 kV for sample analysis. The obtained SEM images were further analysed using ImageJ software to assess the size and structural characteristics of the cookies.

Table 2: Ingredient percentage per 100 g of cookies

Ingredients	BCFM 11	BCFM 12	BCFM 13	BCFM 14
Whole wheat flour (Atta)	52.52	19.09	27.50	25.38
Pea protein Isolate	0.00	31.56	0.00	0.00
Soy protein isolate	0.00	0.00	23.96	0.00
Whey Protein isolate + Skim milk powder	0.00	0.00	0.00	25.78
Almond bits	0.00	3.70	3.73	3.74
Palm oil	26.26	25.33	25.53	25.58
Sugar	20.20	20.26	20.43	20.46

Dry Creaming – whole wheat flour, Sugar, Palm oil, emulsifiers and flavors were added to the mixer (Bismac) and mixed for 3 minutes

↓

Wet Creaming – Salt, Sodium bi carbonate and ammonium bi carbonate were dissolved in water and added to the mixer and mixed for 3 minutes

↓

Mixing – The composite flour mix added to the mixer and slow mixed for 4 minutes

↓

Dough unloaded from mixer and rested for 20 minutes in a trolley, Dough extruded into cookies shape

↓

Each batch was baked at 175°C in a deck oven for 18 to 20 minutes (National, Japan).

↓

Cookies cooled at room temperature for 20 minutes and packed. All combinations were prepared in triplicate with 12 per combination mix prepared for analysis

Figure 1: Flowchart for the preparation of high protein cookies

2.7 X-Ray Diffraction analysis

The crystallographic structure of the cookies was investigated using X-ray diffraction (XRD) analysis with a Rigaku Smartlab (Japan), available at the Research Lab, University with Potential for Excellence (UPE), University of Mysore, Karnataka, India. The analysis was conducted at a power level of 3 kV, with samples positioned in a rotating anode set to 60 A. The system utilized a HyPix-3000 high-energy resolution 2D HPAD detector. The resulting XRD curves were analyzed using Match software to determine the degree of crystallinity, as well as the 2θ and d values for all four samples.

2.8 Sensory Analysis

Descriptive sensory analyses with eighteen panellists were performed for the final products. Smaller group was chosen as trained experts provide more reliable and consistent results than a large group of untrained consumers. The hedonic evaluation of the cookies' sensory attributes which included appearance, aroma, taste, texture, and after taste and overall acceptability were done and mean values were noted. Informed consent was secured from all participants in the sensory evaluation, comprising both male and female individuals aged 25 to 56 years. They received thorough information about the study's objectives, methods, and any potential risks associated with their participation.

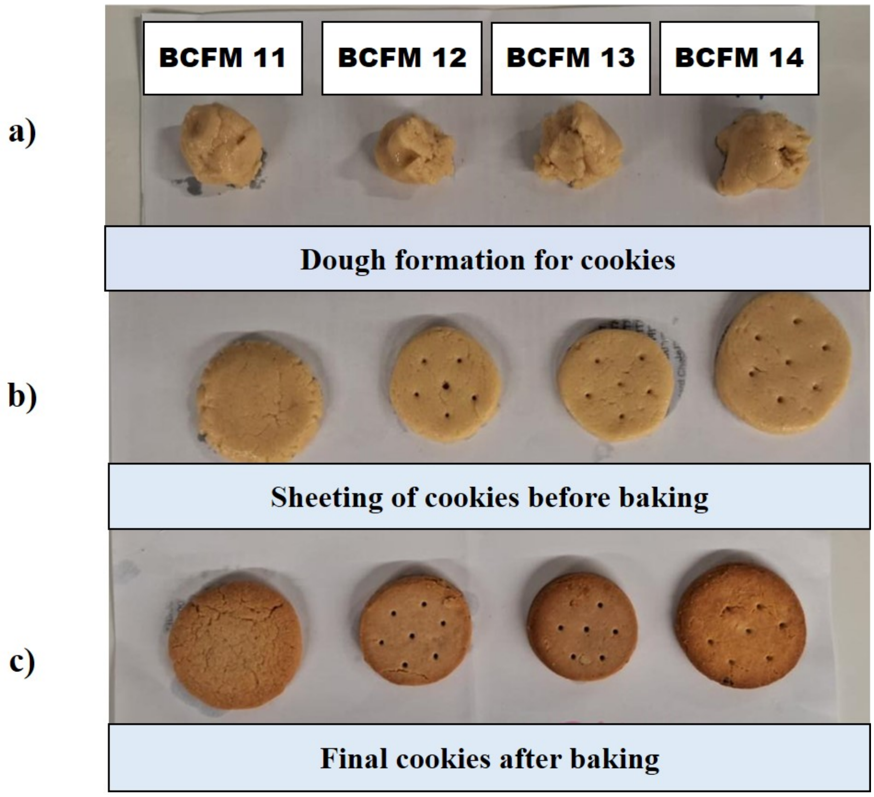

Figure 2: Schematic representation of making of cookies. a) Formation of dough with all four combinations mix for making of cookies; b) The dough is sheeted before baking the cookies; c) The cookies were formed after baking process

2.9 Statistical analysis

The sensory analysis data were subjected to ANOVA t-test and to verify the experimental data were statistically significant. The graphs were plotted using Microsoft excel software (version 2016).

3. Results and Discussion

3.1 Rheological properties of Composite flour mix

The Farinograph studies demonstrate that incorporating protein into wheat flour has a significant impact on dough development time, water uptake, and baking qualities of the final products (Table 3). Water retention is 48.5% in the BCFM 12 combination, which may be attributable to the inclusion of protein sources leading to high water retention property [11].

Table 3: Farinograph results of Cookie formulations

Parameters	BCFM 11 (Control)	BCFM 12	BCFM 13	BCFM 14
Consistency	490	420	468	688
Water Added	85	52	48	47
Water absorbed default consistency	81	48.5	46.3	46.2
Water Absorbed for default moisture	76	44.3	42.5	41.2
Development time	4.9	15.3	12.9	17.9
Stability	4.1	3.3	0.3	1.2
Time to break down	9	18.6	13.2	19.1

Results from this study showed that the dough development time of BCFM 12 has increased from 4.9 to 15.3 (Fig 3). The other protein combination resulted in a substantial increase in dough development time against the control sample. The inclusion of protein rich sources increased the development time and dough stability. The increased DDT duration might be associated with gluten protein dilution, which are comparable with other studies [12].

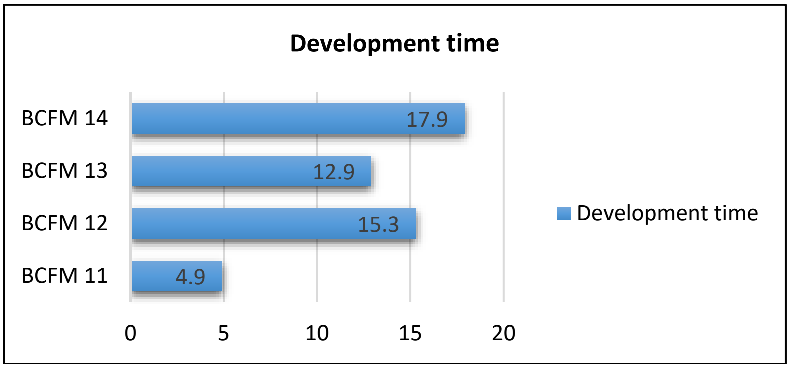

Figure 3: Graph representing the dough development time of all combination mix

The dough quality number reflects the dough's durability and capacity to stand up to kneading. The quality number for BCFM 14 was around 109 when compared to the control. Though the quality number was high for BCFM 14, the machinability to mould the dough was better in control (BCFM 11) with whole wheat flour for which the quality number was 90. A higher quality number shows that the flour strength increases with the addition of a protein source to the wheat flour mix, which is distinguished by prolonged stability, a limited degree of softening, and a lengthy development time[13]. The decline in quality number might be linked to gluten-gluten associations in the control sample.

The stability value signifies the dough's mechanical strength as well as its ability to maintain consistency under shear over time. The stability value of BCFM 12 was around 3.3, with the lowest value of 1.2 for the BCFM 14 combination. The most significant stability value suggests that the inclusion of high protein reinforced the dough structure and increased its durability under shear [14].

The tolerance index value is the evaluation of the degree of resistance and tolerance that exists before weakening the dough network, which is expressed in farinograph units (FU). BCFM 12 combination showed the highest Tolerance Index value of 86 compared to the control of 40, while the lowest value was identified in the control BCFM 11, which was about 40. Lower tolerance numbers are suited to products where short mixing times and a more delicate gluten structure are ideal, but the high protein combination with the highest TI value has poor dough handling features especially for making cookies [15]. The inclusion of soy, Pea and whey isolate proteins lead to denser and firmer dough compared to using whole wheat flour alone. This is because soy, pea and whey proteins lack gluten but interact with the gluten matrix, contributing to an overall stronger dough structure but results in poor machinability to form cookies.

The breakdown time of the control sample was lower, about 9 minutes, and rapidly increased to 18.6 minutes for BCFM 12, with the maximum value reported in BCFM 14 at 19.1 minutes (Fig.4). The breakdown period has a significant impact on the pace of breakdown and strength of the flour mix. The higher the breakdown time, the more fragile the flour mixture [16].

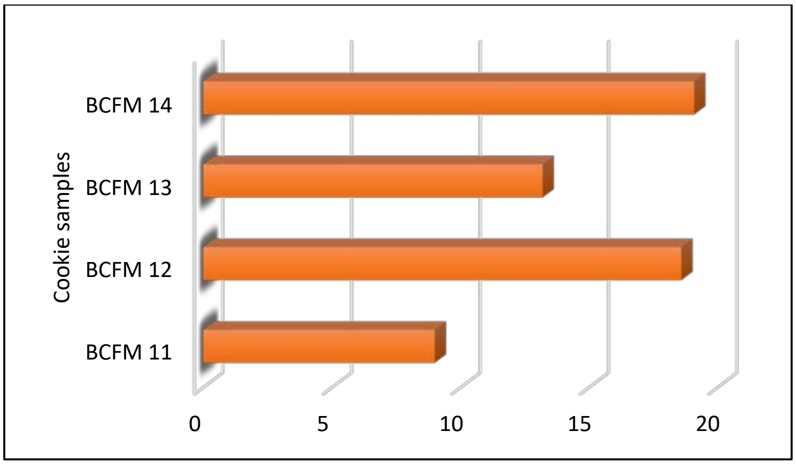

Figure 4: Graph representing the breakdown time of all combination mix

1) BCFM 11 (Control)

Dough Development Time (DDT): 4.9 minutes

Stability: This formulation serves as the baseline for comparison, exhibiting moderate stability. The lower DDT suggests that the dough reaches optimal consistency relatively quickly.

Implications: The control mix has good handling characteristics, making it suitable for standard cookie production. However, it lacks the nutritional enhancements found in the other formulations, which may not meet the growing demand for healthier snack options.

2) BCFM 12 (Atta + Pea Protein Isolate)

Dough Development Time (DDT): 15.3 minutes

Stability: This formulation shows a significant increase in stability due to the high protein content, reflected in a high tolerance index (86). The longer DDT indicates that the dough requires more time to reach optimal elasticity.

Implications: While BCFM 12 enhances the protein profile of cookies, its longer DDT and increased stability may reduce flexibility, potentially making it less suitable for specific cookie types that require pliability. The high protein content also contributes to strong antioxidant potential, adding health benefits.

3) BCFM 13 (Atta + Soy Protein Isolate)

Dough Development Time (DDT): 12.9 minutes

Stability: This formulation has a stable dough due to the inclusion of soy protein, with a DDT that is shorter than BCFM 12, indicating a somewhat quicker development time.

Implications: BCFM 13's favorable DDT and stability suggest it may be suitable for cookie production while offering nutritional benefits from soy protein. The moderate moisture content also suggests potential for extended shelf life, enhancing its commercial viability.

4) BCFM 14 (Atta + Whey Protein Isolate + Skim Milk Powder)

Dough Development Time (DDT): 17.9 minutes

Stability: BCFM 14 exhibits the highest DDT, indicating that the dough requires significant mixing time to develop. However, it also shows strong stability due to the combined effects of whey protein and skim milk powder.

Implications: Although BCFM 14 may be more challenging to handle due to its longer DDT, it offers enhanced nutritional value. The high protein content contributes to its appeal as a healthier option, but the longer DDT may require adjustments in production methods to improve dough manageability.

3.2 Proximate analysis of composite mix

3.2.1 Moisture

The moisture level of food has an impact on its sensory, physical, and structural qualities. Microbial development is triggered by high moisture content, while food material's ability to be stored is impacted by low moisture content [3]. The moisture content affects the texture of the cookies; a low moisture dough results in burned cookies, while a high moisture level affects the crunchy texture of the cookies [17]. The moisture content of BCFM 13 (soy protein isolate mix) was determined to be 8.9% (Table 4) when compared to the control value of 10.2% [5]. The high moisture content of the BCFM 13 may be attributed to the better moisture retention capacity of the soy protein [18] and also indicating that the final product would last for longer periods [3].

3.2.2 Ash

The ash content in all combinations increased compared to control. The BCFM 14 had the highest value of 3.1% (Table 4) which were comparable to the studies of Varghese et al [3] and Wani et al [19]. Higher ash levels suggest that the protein combo cookies contain more minerals [17]. The variation in ash concentration across all combinations is attributable to the inclusion of protein sources in the flour mix.

Table 4: Proximate composition of composite flour mixes

Composite Combination	Moisture (%)	Ash (%)	Fat (%)	Protein (%)	Carbohydrate (%)	Dietary Fibre (%)
BCFM 11	10.2	1.4	2.1	12.1	76	10.1
BCFM 12	7.2	2.5	3.7	53.22	32.5	7.8
BCFM 13	8.9	2.3	4.2	46.76	40.2	8.5
BCFM 14	7.9	3.1	0.9	49.92	35.9	5.9

3.2.3 Fat

The fat contents of all cookie flour combinations was found to be substantial, with the highest value of 4.2% in BCFM 13 (Table 4) whereas soy flour replacers enhance the fat content of the final cookie product.

3.2.4 Protein

The protein content of all combinations had increased as the protein source has been included to give a nutritious meal for the children. BCFM 12 encompassed a high protein level of 53.22%, whereas the final product contained 24.17% protein (Table 4) [20]. The protein content of starch granules has a significant impact on cookie development because proteins denature and become more difficult to expand. Without proteins, starch granules are readily broken, raising water content. Fats may collaborate with starch granules to increase viscosity and prevent water penetration [17]. During baking of dough, the dough goes through the glass transition gaining mobility that allows it to interact and form a web which influences the flow of the cookies during baking [21].

3.2.5 Carbohydrate

The carbohydrate level of the combinations declined when compared to the control due to the addition of protein sources into the flour mix, with the BCFM 12 combination having the lowest carbohydrate content at 32.5% (Table 4).

3.2.6 Dietary fibre

The dietary fibre content of the BCFM 13 was determined to be 8.5%. According to Hayat et.al [4] findings, it has been contended that the removal of the bran and aleuronic layer of the wheat during the milling process contributes to the flour mix's relatively low fat and fibre content.

3.3 Microstructural analysis of cookies

Figure 5 provides information that reveals the cookie's porous structure, characterized by several interlinked pores of different sizes. The presence of trapped air during the baking process is a possible cause of this porosity, which enhances the texture and lightness of the cookie. Analysing the BCFM 12 sample indicates an intricate interplay between the wheat flour and soy protein constituents. The presence of uneven forms and different densities suggests a diverse distribution of these elements. We can observe starch granules, a distinctive feature of wheat flour, as minute, circular structures embedded in the matrix.

The concentration and processing conditions of soy protein can influence its potential to form aggregates or clusters. These aggregates may manifest as more compact sections within the cookie's structure. The SEM research revealed that the porous structure of the cookie and the interaction between ingredients likely influence its entire texture, including its crispness or chewiness. The allocation of wheat flour and protein sources can have an impact on the cookie's taste characteristics. The presence of carbohydrate and protein can influence the nutritional value of the cookie.

3.4 Crystallographic Study

The crystallinity degree in the control and BCFM 14 exhibits a relatively similar degree of variation, as evidenced by Table 5. However, BCFM 12 and BCFM 13 exhibit a slight increase in crystallinity. The final biscuits were classified as moderately crystalline based on the values of BCFM 11 and BCFM 14. The degree of crystallinity for BCFM 12 and BCFM 13 is not significantly different (18.34% and 18.97%). These cookies would provide the end user with a mouthfeel that is both tender and crisp. The overall texture and shelf life of these cookies are influenced by their ability to retain moisture.

The primary component of wheat flour is starch, which is the primary cause of crystallinity in Cookies. Amorphous starch is more disordered, whereas crystalline starch has a more organized structure. The texture, moisture content, and shelf life of biscuits can all be influenced by the degree of crystallinity. The XRD analysis of BCFM 12 (wheat flour with pea protein cookies) was similar with the results of Purohit et al [22] where the cookies have a crystalline intact structure. The 2θ in degree peaks in all four combinations likely indicates the presence of amylose which influences the spread ability and thickness of the dough as it contributes in the viscosity of the dough. The pea protein interaction with amylose was crucial where the pea protein provides the matrix for the interaction of the starch that results in stable and cohesive structured cookies. During baking process, amylose forms a gel which provides strong framework of the cookies [23]. In terms of storage, the presence of amylose would provide a crumby texture due to retrogradation which provides a longer shelf life of cookies.

3.5 Sensory analysis

Eighteen panellists performed the sensory analysis of the final products. Fig.6 shows the hedonic average evaluation of the cookies' sensory attributes, which includes appearance, aroma, taste, texture, and after taste and overall acceptability of cookies. BCFM 14 scored the highest (8.3) for appearance, while BCFM 13 received the lowest score (7.5). When cookies are baked, enzymatic browning has a major impact on their appearance [13].

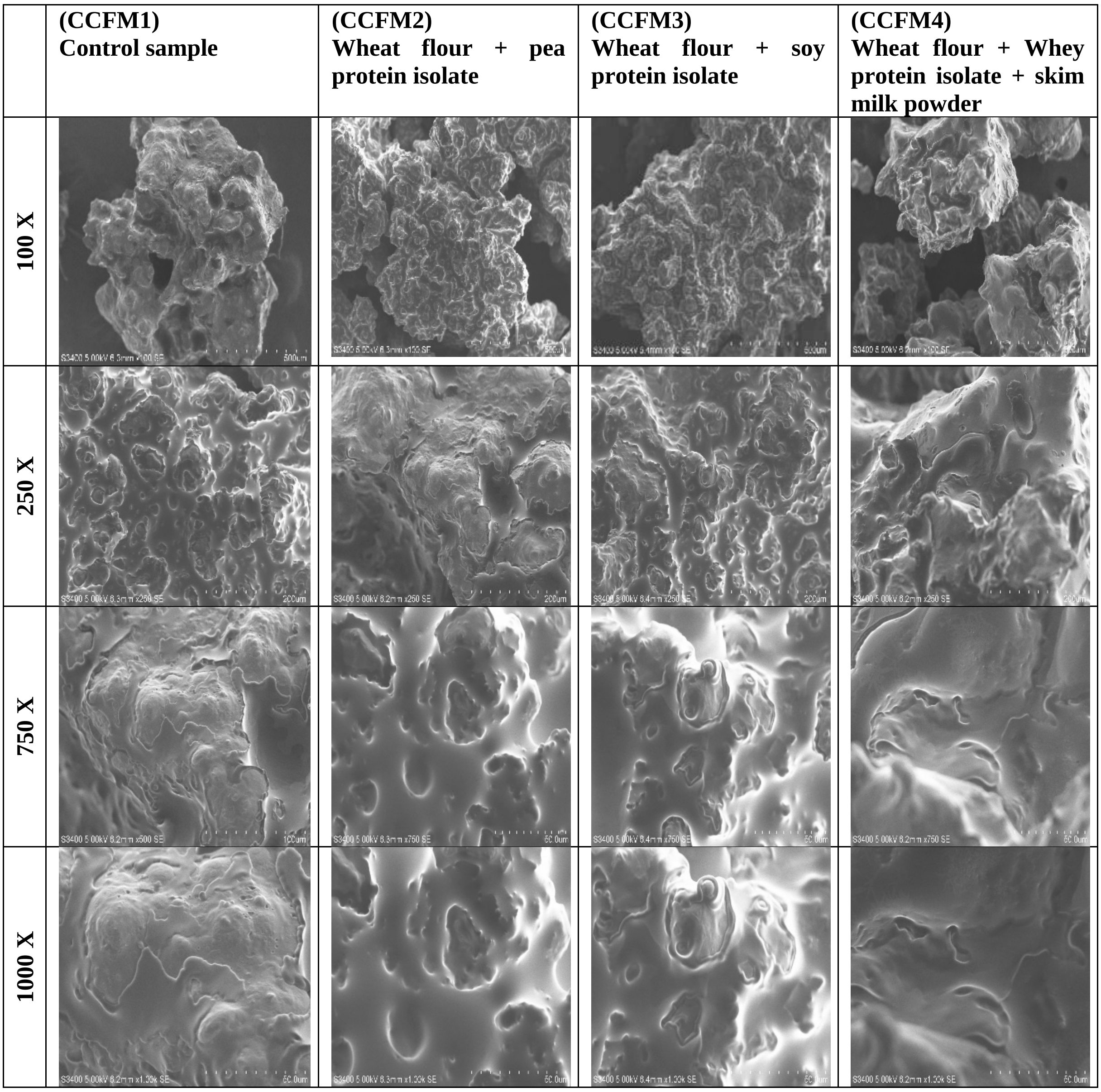

Figure 5: SEM images of the cookies samples at different magnifications (100X, 250X, 500X, 750X and 1000X)

Table 5: Crystallographic Study of composite flour mixes

Samples	2θ in degree	d [Å]	Degree of crystallinity (%)
BCFM 11	19.42^0 20.46^0	Min- 5.0113 Max - 1.3348	15.48
BCFM 12	20.18^0	4.3968	18.34
BCFM 13	20.36^0	4.3589	18.97
BCFM 14	Min- 18.44^0 Max- 39.50^0	Min- 4.8086 Max – 2.2795	15.54

The three main components of cookie flavour-aroma, taste, and aftertaste—all have a strong correlation with small-molecule aromatic chemicals that are detected by chemical pathways and sensory receptors. Each of the three metrics had comparable patterns, with BCFM14 exhibiting the highest value of 8.7. The possibility that some aromatics were trapped and not released into the saliva accounts for the other combinations' lowest flavour score. The textural profile had a significant impact on cookies' palatability with a value of 9 for control sample received followed by BCFM 14 with a value of 8.5 and BCFM 13 with a value of 8 [24].

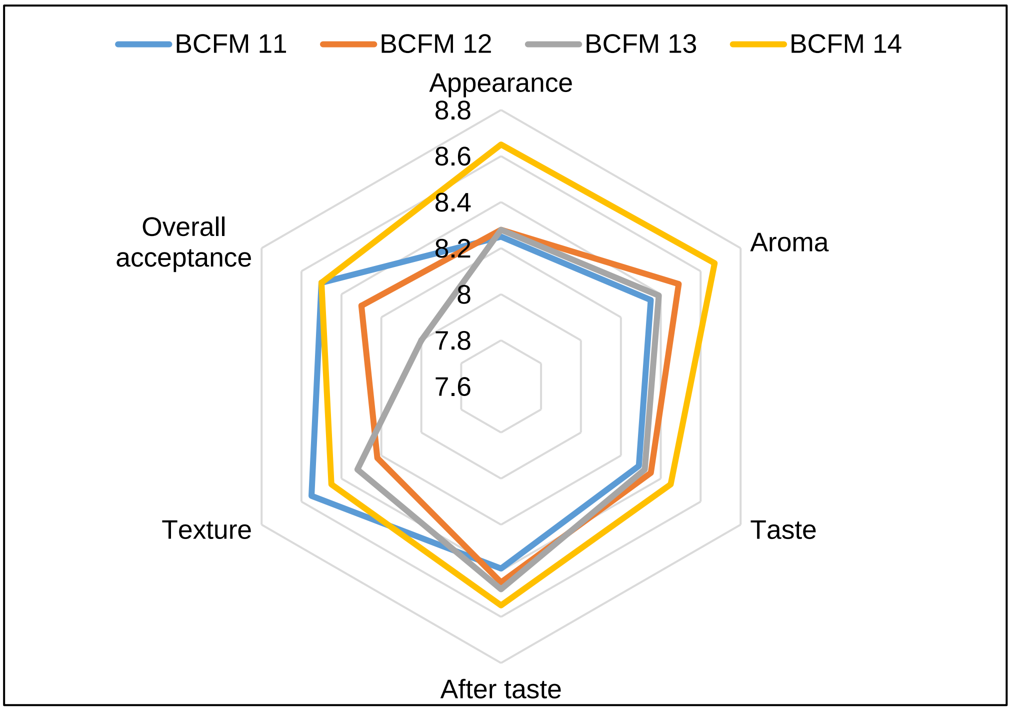

Figure 6: Graph representing the sensory analysis of Cookies

All samples had a sufficiently favourable and higher response from the panellists in the context of general acceptability, with scores ranging from 8 to 8.5. BCFM 14, among the high-protein combinations, obtained the highest score of 8.5. Following BCFM 14, BCFM 12 was the next recommended sample, and it backed up the lowest score. The panellists like the combination of whey protein and skim milk powder the most, maybe because of the mouth feel, colour, and texture [25].

3.6 Statistical analysis

From the ANOVA t-test, the mean values of all four samples have significant difference from the experimental values indicated by the p-values (all less than 0.05). The results suggested that the means of all the samples are not equal to 0 and they are statistically significant. The Table 6 results confirm the significant deviation at 95% confidence intervals from the test value which offers a mean difference.

Table 6: Statistical analysis for the sensory analysis of the cookie's samples

Samples	**t**	**df**	**Sig. (2-tailed)**	**Mean difference**	**95% Confidence Interval of the Difference**	**Samples**
					Lower	**Upper**
BCFM11	381.000	5	.000	9.52500	9.4607	9.5893
BCFM12	97.000	5	.000	8.08333	7.8691	8.2975
BCFM13	37.786	5	.000	7.58333	7.0674	8.0992
BCFM14	39.590	5	.000	6.08333	5.6883	6.4783

Note: * The **t-statistic** is the ratio of the departure of the sample estimate from the null hypothesis value to its standard error. It measures how far the sample data is from the null hypothesis in standard error units.
**** Degrees of freedom (df)** refer to the number of independent values in a data set that can vary while still satisfying a certain constraint (like the mean or variance).
***The **p-value** or **Sig. (2-tailed)** is the probability of obtaining test results at least as extreme as the ones observed, assuming the null hypothesis is true.

4. Conclusion

The evaluation of composite flour mix formulations shows that BCFM 12 (Atta + pea protein isolate) and BCFM 13 (Atta + soy protein isolate) notably enhance the nutritional and functional properties of cookies. BCFM 12 stands out for its increased protein content, contributing to a healthier nutritional profile with benefits such as improved muscle maintenance, satiety, and blood sugar management. BCFM 13 has higher moisture content which aids in extending the shelf life of baked goods, though its high protein content may reduce dough flexibility, possibly limiting its suitability for cookie production.

Scanning Electron Microscopy (SEM) analysis of BCFM 12 reveals insights into structural and textural improvements, showing that pea protein and wheat flour interactions enhance cookie texture and lightness through increased porosity. X-ray diffraction (XRD) data indicates moderate crystallinity in both BCFM 12 and BCFM 13, a factor that contributes to a balance of tenderness and moisture retention, ultimately improving cookie stability and appeal.

Sensory evaluation highlights BCFM 14 (Atta + whey protein isolate + skim milk powder) as the most preferred formulation for appearance, flavor, and overall acceptability. Statistical analysis using ANOVA confirms significant differences in sensory attributes across all samples, with p-values below 0.05.

In summary, these findings suggest promising potential for high-protein, nutritionally dense cookies, with positive implications for public health. Future research may focus on optimizing protein ratios within Atta mixes to balance nutritional benefits with improved dough machinability.

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