International Journal of Environmental Sciences
ISSN: 2229-7359
Vol. 10 No. 4, 2024
https://theaspd.com/index.php

Development And Characterization Of Flaxseed Protein-Enriched Cookies

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Abstract

Cookies are regarded as an appropriate source of nutrients including carbohydrates and fats and can be enriched nutritionally by enriching them with proteins by partially replacing the wheat flour with protein isolates. The objective of this study was to determine the best incorporation level of flaxseed protein in cookies that enhance the nutritional and sensory properties. Conventional extraction methods were used to isolate flaxseed protein that was subsequently modified with the use of microwave. The reason why flaxseed meal was chosen as a possible protein source is because it has a high biological value according to the literature. The refined wheat flour was replaced in this study with modified flaxsed protein isolates in varying concentration (0.5%, 1%, 1.5 and 2%), and control formulation. The cookies were assessed in terms of physical, chemical, textural and sensory characteristics. Of the formulations that were tested, cookies with 2 percent flaxseed protein had better textural properties and acceptable attributes. The research arrives at the conclusion that flaxseed protein can be used as a useful functional ingredient in bakery recipes to increase the protein content and the nutritional value of such recipes.

Keywords: *cookies, flaxseed, protein enrichment, functional ingredients, protein isolates, flaxseed meal*

1.0. INTRODUCTION

Flax, which is also known as Linum usitatissimum, is a plant that is of the family Linaceae, and it is considered as one of the oldest crops that have been cultivated due to its versatility and versatility in usage. The plant is slender with fibrous stems and has blue flowers that bear capsule fruits in the form of spheres up to 6080 cm high every year. The capsule has two parts and its seeds are usually referred to as flaxseeds or linseeds, which are flat and oval, have a glossy surface, a pointed end, and are accompanied by a nutty taste. Flaxseeds are usually between 3.0 and 6.4 mm, 1.8 and 3.4 mm, and 0.5 and 1.6 mm in length, width, and thickness, respectively (Yilmaz and Altuntaas, 2020). In the past, flax was utilized in the textile industry to produce linen, wraps to cover mummies in Egypt and household fabric via spinning and weaving (Karg et al., 2024). Produced worldly, in recent decades, the flaxseed has been severely growing, to about 4.2 million tons in 2022, a 17% growth since 2020-21. The major producers are Russia and Kazakhstan, with almost 66 percent of the world production, and 10.5 percent is produced by India (Selge et al., 2024). Flaxed oils, protein, and dietary fiber constitute rich nutrients with a nutritional content of 30, 20 and 30 percent respectively (Marambe and Wanasundara, 2017). Its oils are rich in unsaturated fats with 12-30% oleic acid, 8-29% linoleic acid and 35-67% 0-linolenic acid a vital omega-3 fatty acid also present in fish (Al-Madhagy et al., 2023). Flaxseed oil can be used in the industrial sector since it is a high-linolenic acid and is extensively applied in paint, varnishes, and coating industries.

The by-product of oil extraction known as defatted flaxseed meal is usually used as animal food even though it is very nutritious and has the potential to benefit human health. The meal is high in protein, phenolic compounds, and lignans that help to enhance its antioxidant property (Lorenc et al., 2022). Also, flax seed has 5-8% mucilage fiber, which offers high water-holding characteristics and is similar to gum arabic making it useful in the addition of many food systems (Lorenc et al., 2022). Flaxed seed has also been taken in the past in a medical aspect to relieve cough, stomach aches, and inflammation. It possesses anti-inflammatory, analgesic, and antipyretic effects which can be attributed to its bioactive compounds (Arslanoğu and Aytaç, 2020; Ishag et al., 2019). Although these are the advantages, the application of flaxseed in food processing is low because extraction of its protein is not straightforward. Its seed coat polysaccharides are also mucilaginous, which swell in solvents and viscosity rises as well as decreased protein recovery (Puligundla & Lim, 2022). The traditional techniques of solubilizing proteins include alkaline solubilization (pH 79) which increases protein solubility but necessitates a great amount of water and can lead to suboptimal yields (Kheto et al., 2024). Pretreatments such as physical dehulling enhance the protein recovery with about 35-40 percent protein content of defatted flaxed seed meal

(Kheto et al., 2024). Most recent developments have been pointed at microwave pretreatment, which was reported to degrade polysaccharides, inactivate lipase enzymes, enhance flavor, and stimulate protein retention by changing molecular structure and increasing solubility (Xu et al., 2020). These technological advancements are imperative in increasing the use of flaxseed as an eco-friendly, non- meat, protein source. The bakery items form a significant industry in food industry since they have a long shelf life, are economical to the consumer, and can be easily accepted by the consumer. They give a good opportunity of nutritional fortification through the addition of functional ingredients. The country of India is second in the market of biscuits and cookies after the United States. Addition of protein isolates to bakery products can greatly improve the nutritional qualities of such products that are generally consumed widely. Given that about 55 percent of bakery products are eaten in rural societies and 37 percent of the bakery products are eaten by the low-income earner, fortified bakery products can be used in controlling protein deficiency especially in the vegetarian dominated societies. The study will be done to create and maximize the protein-enriched cookies with the use of flax seed protein isolates and study the effect of microwave-modified flax seed protein on the physicochemical, textural as well as the sensorial properties of cookies. The findings are expected to contribute to the formulation of nutritionally superior, affordable, and plant-based bakery products catering to the growing demand for sustainable protein sources in the food industry.

2.0. METHODOLOGY

2.1. Procurement of raw materials

Flaxseed (*Linum usitatissimum*) LC-2063 were procured from PAU, Ludhiana, Punjab. Soybean oil was purchased from local market of Baru sahib for the preparation of oil in water emulsions. All other chemical used in this study were available in the Department of Food Technology, Dr. Khem Singh Gill Akal College of Agriculture, Eternal University, Baru Sahib, District Sirmaur, HP.

2.2. Preparation of flaxseed meal

Oilseed meal was extracted using the whole raw flaxseed by the method given by (Sharma et al., 2019; Tan et al., 2016) with minor modifications. The seeds were finely ground using mortar and pestle and then sieved properly to remove the coat. The flaxseeds were further soaked in distilled water (1:18) at 60^{o}C for 3 h. The de-mucilage flaxseeds were then dried in hot air oven at 45^{o}C for 24 h and then further grind. Three different method were employed for the extraction of oil from both castor seeds and flaxseeds which included conventional method where oilseed powder and mixed with n-hexane (1:10) at 55^{o}C at 175 rpm for 4 h, soxhlet extraction with the help of petroleum ether and enzyme-assisted aqueous extraction with the help of 2% w/v pectinase enzyme followed by freeze drying of the meal for the extraction of protein.

2.3. Preparation of protein isolates and its modification

The different meals obtained from above mentioned methods was then further crushed and de-fatted using hexane (1:6) for 3 h. The obtained powder is then mix with 0.1 M tris-buffer with the pH 8.6 in the ratio of 1:10 with regular stirring for 3 h. The mixture is then subjected to centrifuged at 10000 rpm for 10 min followed by separation of residue using cheesecloth. The pH of extracted supernatant was then again adjusted to 4.2 using 0.1 M Hcl for the precipitation of oilseed protein followed by centrifugation and precipitate was then stored at 4^{o}C. Lypholyzation of the collected precipitate was then carried out for the drying followed by grinding and storage at -20^{o}C (Kaushik et al., 2016). Three different meal were utilized for the extraction of protein namely protein extracted from conventionally defatted meal, protein extracted from soxhlet defatted meal and protein extracted from enzymatically defatted meal. Microwave assisted modification of protein was carried out using the method given by (Bandyopadhyay et al., 2012) with minor modifications. The microwave treatment was given at a slurry ratio of 1:10, for 50 seconds at 450W. The obtained protein samples were then freeze-dried, followed by grinding and stored in an air-tight container for further experimentation. Three types of modified protein were obtained, namely modified protein from conventionally extracted flaxseed meal, modified protein from soxhlet extracted flaxseed meal and modified protein from enzymatically extracted flaxseed meal, respectively.

2.4. Development of recipe for the preparation of protein enriched cookies

The formulation of protein-enriched cookies was standardized using refined wheat flour as the base ingredient, partially substituted with modified flaxseed protein isolate. Other essential ingredients included shortening, sugar, leavening agent, coconut essence (as a flavouring agent), and water. The

addition of flaxseed protein was done to increase the protein level of the cookies without compromising the desirable sensory and textural characteristics.

Table 1 shows the standard recipe and ingredient formulation of the control and flaxeed protein-based formulations. The four formulations (F1 to F4) were made by the substitution of refined wheat flour with different concentrations of modified flaxseed protein (1.5 to 2.5 percent) and the control formulation was made of refined wheat flour only. The cookies were made based on the standardized process outlined by Soni et al. (2018) in line with the process flowchart in Figure 1.

Table 1. Standardized recipe for formulation of cookies incorporating modified flaxseed protein

Ingredients (g)	Control	F1	F2	F3	F4
Refined Wheat flour	25	24	23.5	23	22.5
Flaxseed protein	-	1.5	1.5	2	2.5
Shortening	12.5	12.5	12.5	12.5	12.5
Sugar	12.5	12.5	12.5	12.5	12.5
Leavening Agent	0.1	0.1	0.1	0.1	0.1
Water(mL)	5	5	5	5	5
Flavour (Coconut essence)	1	1	1	1	1

The weighing of the ingredients was then done with accuracy according to the formulation. Creaming was done with all the shortening and sugar until a light, fluffy, and homogenized texture was achieved. Refined flour, leavening agent and flax protein isolate were also dry ingredients and sieved and slowly added to the creamed mixture. The dough was kneaded uniformly with the addition of measured water and flavoring agent to achieve the desired consistency. The prepared dough was rolled to a uniform thickness, cut into circular shapes, and baked in a preheated oven at 180 ± 2°C for 15–20 minutes until a golden-brown appearance was achieved. After baking, the cookies were cooled at room temperature, packed in airtight containers, and stored for further analysis.

Figure 1 Flow chart for preparation of cookies

2.5. Sensory evaluation

The cookie samples prepared were subjected to sensory analysis by the panel using 9-point hedonic scale (Soni et al., 2018). Score was given on the score card. Based on the average sensory results, cookie samples were streamlined for the best overall acceptability which is better for incorporating castor and flaxseed-based proteins.

2.6. Physico-chemical analysis

The prepared cookie samples were incorporated with Soxhlet-extracted modified flaxseed protein at various levels by replacing refined wheat flour at 0.5%, 1%, 1.5% and 2% were analysed were analyzed for physical properties such as texture profile. Chemical analysis of the prepared cookies sample prepared using optimized formulation based on maximum sensory score was carried out using standard procedure (Soni et al., 2018).

2.7. Proximate analysis

2.7.1. Moisture content

The samples were analysed for moisture content by the air-oven drying method (International, 2000): 930.15. The samples were dried in an oven at 103 ± 2°C for 7-8 h or till the consecutive weighing does not vary by more than 3-5 mg. The weight of the samples was taken after cooling them in desiccators.

Moisture % = $\frac{W1-W2}{W1} \times 100$

Where, W1= Sample flour weight before drying

W2= Sample flour weight after drying

2.7.2. Crude Protein

It was determined as per the methodology described by (International, 2000): 981.10 using Kjeloplus (Pelican). This process involved three steps: Digestion, distillation, andtitration. For digestion, the sample will be weighed in the digestion flask and the sample willbe heated in the presence of sulphuric acid, anhydrous sodium sulfate and a catalyst (copper, selenium, titanium or mercury) till the sample is digested. Once the digestion of the sample is completed neutralization of the sample will be conducted. The digested sample present in the digestion flask will be converted into alkaline by adding sodium hydroxide which further converted the ammonium sulfates into ammonium vapours. Titration is carried out to estimate the presence of nitrogen content in the sample.

Protein % = $\frac{(A-B)\times N \times 14.007 \times 5.7}{W} \times 100$

Where, A= Volume of Hydrochloric acid used in the sample for titration (mL)

B= Volume of Hydrochloric acid used in blank titration (mL)

N= Normality of HCl used

W= Weight of sample flour (g)

14.07 = Atomic weight of nitrogen present in protein

5.7= Conversion factor

2.7.3. Crude Fat

Crude fat was estimated as per the method described by (International, 2000) by equipment Soxoplus (Pelican) using acetone as an organic solvent. 250 mL of petroleum ether was added to extraction flask followed by insertion of flask in extractor. Process of fat extraction took 6 h after which, flask was removed and the solvent remained was evaporated at 110°C. The extraction flask was then cooled to room temperature by placing in dessicator and then weighed. Crude fat content was measured as follows:

Crude fat %= $\frac{Weight\ of\ extracted\ fat}{Weight\ of\ the\ sample\ taken} \times 100$

2.7.4. Crude Fiber

Crude fibre content was estimated as per the method described by (International, 2000):948.22 by equipment Fibroplus (Pelican equipment). The moisture and fat free samples were measured in the amount of 3g and transferred in empty Gooch crucibles. H_2SO_4 (1.25%) was poured to the sample and the content was heated at 380°C for 90 min. Filtration of the sample was carried out with the help of suction followed by distilled water washing of samples. All the content was again reacted with NaOH (1.25%) solution followed by again heating at 380°C for 90 min. The obtained content was again filtered out and washed followed by drying at 130°C, cooling at room temperature and weighing. The crucible comprising sample were then placed in muffle furnance at 550°C for 4-5 h. Crucible were then kept in

dessicator to cool them to ambient temperature and then weighed again. Crude fiber of the sample was measured using the equation:

Crude Fiber (%) = $\frac{W1-W2}{W} \times 100$

Where, W is weight of fresh sample

W1 is the crucible weight + sample before ashing

And, W2 is the crucible weight + sample after ashing

2.7.5. Ash content

The total ash content of all samples was estimated by the method explainedby (International, 2000). The samples were quantified in silica crucibles and ignited at 550°Cfor 4-6 h, or overnight if needed, in a muffle furnace. Then the crucibles were placed in dessicator to cool them down to ambient temperature. The ash content of the samples was evaluated as follows:

Ash % = $\frac{Weight\ of\ ash}{Weight\ of\ sample} \times 100$

2.7.6. Carbohydrates

Total carbohydrate content of raw and processed underutilized grain cultivars was estimated using the methodology described by (Energy & Requirements, 1973) as per the following equation:

Carbohydrate (%)= 100 -[Moisture (%)+ Protein(%)+ Fat(%)+ Ash(%)+Crude fiber (%)]

2.7.7. Colour characteristics (L, a, and b values)

Color attributes were evaluated by chromameter (CR- 400, Konica Minolta, Japan) at Eternal University, Baru Sahib. The colour of all the samples involved in the present investigation were measured in the form of L*, a*, and b* values using a chromameter. The colour estimation was calculated with the use of colourimetric parameters as per the following equations given by (Zhang et al., 2019). Determination of the color was done using the formula

$$\Delta E = \sqrt{(L^* - L_o).^2 + (e^* - a^o).^2 + (b^* - b_o).^2}$$

Where,

The term indicated Hunter L* i.e. lightness ranging from 0-100, indicating black to white. The value of a* denoted green (-) to red (+) whereas the value of b*represented blue (-) and yellow (+). ΔE represented the colorimetric difference, C* represented chroma, H_a represented hue angle, the browning index was represented by BI and the total colour was denoted by E*.

3.0. RESULTS AND DISCUSSIONS

3.1. Chemical Composition of Flaxseed Protein Incorporated Cookies

Table 2 shows the proximate composition of cookies incorporated with control and flaxseed protein. The samples had significant differences ($p \leq 0.05$) in moisture and protein content and similarity in the rest of the ash, fat, fibre, and carbohydrate contents. With the addition of flaxseed protein, the moisture content was slightly higher in the control (1.43 percent), F4 (1.72 percent), which might have been caused by the greater water absorption capacity of the flaxseed proteins and its hydrophilic character. An increase in the percentage of protein was observed as the inclusion of flaxseed protein increased. The nutritional value of the product was significantly improved as the protein content of cookies was 2.84% in the control and 3.23% in F4. The ash values were in the range of 1.05-1.93% with the highest rate presented in F3 which indicates the presence of mineral constituents that were added by the flaxseed flour. The amount of fat in all formulations was statistically similar ($p > 0.05$) which showed that the incorporation of protein did not affect the fat-holding properties of the dough negatively or the baking process. In line with the same, the fiber content was increasing in a slight but steady manner (3.04-3.49%), which could be explained by the dietary fiber fraction of flaxseed. The content of carbohydrates was 67.55- 68.55% with no significant differences identified in the samples, although a slight reduction was observed as the amount of protein increased presumably as a result of the same proportionate replacement of flour with the protein content. Comprehensively, the addition of flax protein to cookies enhanced the level of protein and fiber content without significantly affecting the fat, ash and carbohydrate content. Formulation F4 revealed the most balanced formulation and the greatest protein enhancement and had acceptable proximate characteristics. This trend of increase in protein content as compared to control was also observed by the study carried out by (Soni, Kulkarni, & Patel, 2018)

Table 2 Effect of Flaxseed Protein Incorporation on the Chemical Composition of Cookies

S. No.	Parameters	Control	F1	F2	F3	F4
1	Moisture content (%)	1.43 ± 0.03^{c}	1.46 ± 0.04^{c}	1.63 ± 0.32^{b}	1.63 ± 0.35^{b}	1.72 ± 0.72^{a}
2	Ash (%)	1.05 ± 0.36^{c}	1.21 ± 0.49^{bc}	1.17 ± 0.33^{c}	1.93 ± 1.25^{a}	1.35 ± 0.21^{b}
3	Protein (%)	2.84 ± 0.64^{c}	2.88 ± 0.21^{c}	3.04 ± 0.21^{b}	3.17 ± 0.26^{ab}	3.23 ± 0.47^{a}
4	Fat (%)	23.09 ± 1.44^{a}	23.71 ± 0.35^{a}	22.46 ± 0.31^{a}	21.67 ± 0.37^{a}	22.31 ± 0.64^{a}
5	Fiber (%)	3.04 ± 0.99^{b}	3.19 ± 0.31^{b}	3.26 ± 0.26^{b}	3.31 ± 0.89^{b}	3.49 ± 0.19^{a}
6	Carbohydrate (%)	68.55 ± 0.16^{a}	67.55 ± 0.26^{ab}	68.44 ± 1.58^{a}	68.29 ± 0.31^{a}	67.90 ± 0.35^{b}

Data are presented as mean ± standard deviation (n = 3). Different superscript letters within a column denote significant differences at $p < 0.05$ according to Duncan's multiple

3.2. Colour Characteristics of Flaxseed Protein–Incorporated Cookies

Table 3 shows the colour features of cookies containing the protein as cookies with flax seeds that are incorporated. The cookies lightness value (L) fell between 53.13 and 65.18 with the control having L 65.18 and the cookies falling within the range of 53.13 and 65.18, meaning that incorporation of flaxseed protein significantly ($p \leq 0.05$) influenced the lightness of the cookies. Lower flax protein cookies especially F1 (65.18) cookies had a lower coloration, and higher protein levels (F3 and F4) had darker surfaces because of higher Maillard-caramelization reaction of reducing sugars and amino acids. The redness value (a) dropped drastically as it was 7.21 in the control, whereas it was 2.18 in F1 which indicated that the addition of flaxseed protein decreased redness and acquiring a dull brown hue which is probably because of the oxidation of polyphenolic compounds and the flaxseed pigments. Likewise, the yellowness coordinate (b*) dropped in the control (20.86) to F1 (16.08) indicating the decrease in the intensity of the yellow due to the alterations in the stability of the pigment and the effect of high protein levels on the browning. A similar trend was observed by (Okpala, Okoli, & Udensi, 2013; Ranhotra, Lee, & Gelroth, 1980) where cookies with lesser protein content were more acceptable.

Table 3. Color characteristics of control and flaxseed protein–incorporated cookies

Parameters	Control	F1	F2	F3	F4
L*	58.43 ± 0.36^{b}	65.18 ± 0.41^{a}	60.31 ± 1.73^{b}	53.13 ± 0.03^{c}	54.19 ± 1.61^{c}
a*	7.21 ± 0.17^{a}	2.18 ± 0.16^{d}	6.10 ± 0.06^{b}	3.81 ± 0.19^{c}	4.29 ± 0.17^{c}
b*	20.86 ± 0.13^{a}	16.08 ± 0.54^{c}	18.36 ± 0.07^{b}	16.19 ± 0.13^{c}	17.54 ± 0.43^{bc}
C*	22.08 ± 0.19^{a}	16.23 ± 0.69^{d}	19.35 ± 0.10^{b}	16.64 ± 0.16^{cd}	18.06 ± 0.47^{c}
Ha	19.08 ± 0.48^{a}	7.72 ± 0.39^{c}	18.39 ± 0.18^{a}	13.26 ± 0.85^{b}	13.77 ± 1.05^{b}
BI	54.43 ± 1.04^{a}	31.38 ± 1.40^{c}	44.73 ± 1.99^{b}	42.50 ± 0.49^{bc}	45.91 ± 0.82^{b}
X	0.40 ± 0.01^{a}	0.36 ± 0.01^{b}	0.39 ± 0.01^{ab}	0.38 ± 0.01^{ab}	0.39 ± 0.01^{ab}
E*	62.47 ± 0.37^{b}	67.17 ± 0.63^{a}	63.34 ± 2.00^{b}	55.70 ± 0.04^{c}	57.13 ± 2.02^{c}

Data are presented as mean ± standard deviation (n = 3). Different superscript letters within a column denote significant differences at $p < 0.05$ according to Duncan's multiple

The colour saturation, denoted as chroma (C*), dropped to 16.23 in F1, as compared to 22.08 in the control, which indicated a paler and dulled coloration in the cookies that had protein in them. The hue angle (Ha) was also significantly different, with the control (19.08) having a yellowish-brown colour and F1 (7.72) having a darker color of brown being observed which means that the hue angle is changing towards a darker colour with the high protein incorporation. The index of browning (BI) dropped at a high rate between the F1 and the control (54.43), indicating that flaxseed protein minimized the intensity of the browning reactions, which is due to the reduced level of sugar and changed matrix structure. There was no significant difference in the X coordinate (0.36 0.40) between treatments indicating that chromatic balance was uniform. The EEE values (total colour difference) were in between 55.70 and 67.17 with F1 exhibiting the most deviation compared to the control which supports the fact that colour differences are visible on the surface. In general, the Multiple Range Test (DMRT) conducted by Duncan revealed that the inclusion of flax protein significantly ($p \leq 0.05$) changed the majority of colour attributes resulting in the darker, less reddish-yellow, and less bright cookies than the control. The moderate amount of flaxseed protein (F2 and F3) produced balanced colour traits with good lightness and browning, but a higher amount (F4) produced darker and dull cookies. Such modifications are mainly related to non-enzymatic browning and pigment reactions during baking, which proves that the addition of flaxseed protein significantly affects the visual characteristics and consumer-friendliness of cookies.

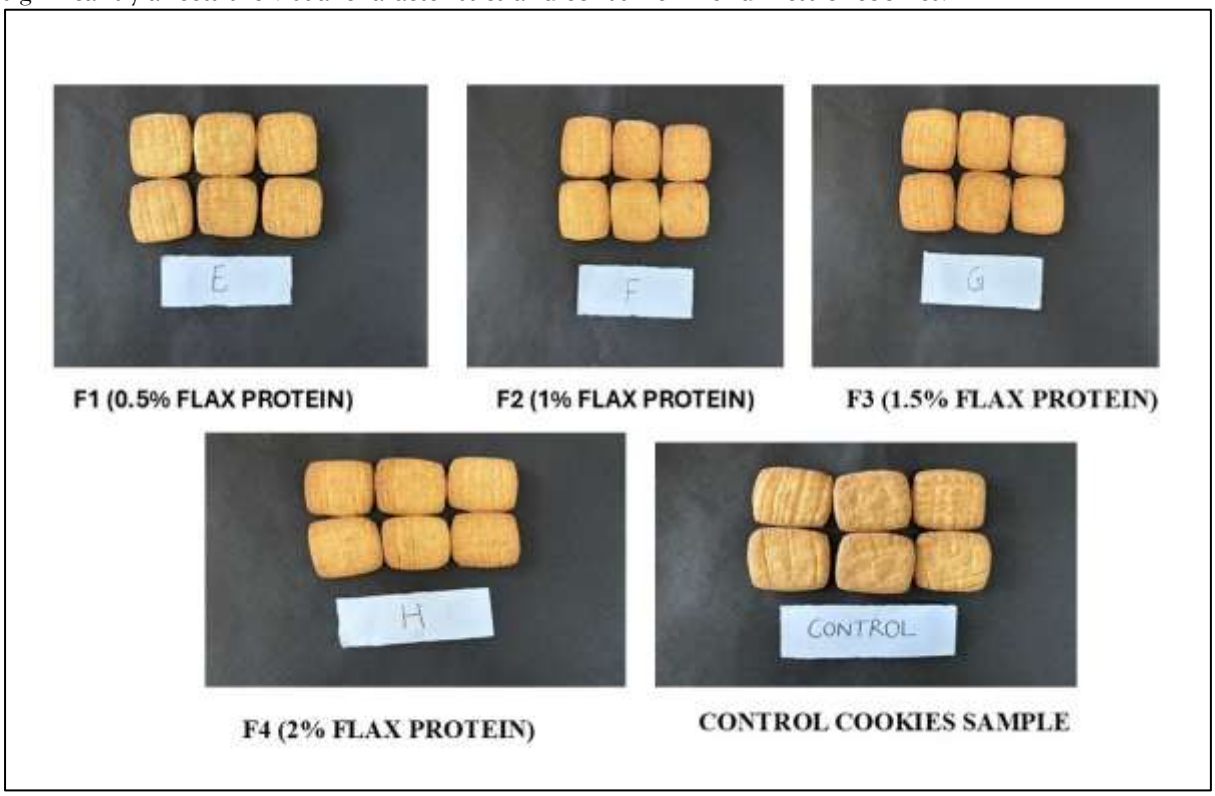

Figure 2: Different blends of cookies obtained through incorporating flaxseed protein isolates in different concentrations

3.3. Sensory Evaluation

The sensory attributes of cookies incorporated with flaxseed protein isolates were evaluated for appearance, texture, colour, flavour, aftertaste, and overall acceptability. Mean sensory scores with corresponding standard deviations and statistical groupings (as per Duncan's Multiple Range Test) are presented in Table 4. Sensory properties of cookies that had flaxseed protein isolates were analysed based on appearance, texture, colour, flavour, aftertaste, and overall acceptability. Table 4 shows mean sensory scores and standard deviations and grouping of the results statistically (according to the Multiple Range Test by Duncan). The protein-enriched formulations F2 (1.5 percent), F3 (2 percent) and F4 (2.5 percent) were not significantly different ($p > 0.05$) in most of the attributes indicating that adding more flaxseed protein than 1.5 per cent did not have a negative impact on sensory quality. These samples constituted an intermediate group (b), which meant similar consumer acceptability. F1 (1%) on the other hand had the lowest scores on sensory, especially on texture, aftertaste and overall acceptability and was statistically

combined as (c). The lack of palatability in F1 may be explained by the inadequate protein addition, which was not able to affect the structural and textural integrity of the product. Flaxseed protein made the surface of the cookies a bit darker as a result of non-enzymatic browning (Maillard reaction) of amino acids and reducing sugars during baking. This effect was, however, within an acceptable sensory range. Protein addition did not have any noticeable impact on the flavour or aftertaste until 2.5 percent, indicating that flaxseed protein can be incorporated successfully without adding bitterness and other unpleasant flavours. In general, F3 (2%) was the most balanced formulation that included the desirable appearance, texture, flavour, and overall acceptability with increased nutritional value. The results are consistent with the findings of Soni et al. (2018) and other studies, which show that moderate concentrations of plant protein addition do not negatively affect the nutritional aspects of bakery goods and do not affect their sensory characteristics..

Table 4 Mean Sensory scores of favoured cookies combination incorporated with flaxseed protein isolates

Sr. No.	Parameters	Control	F1 (1%)	F2 (1.5%)	F3 (2%)	F4 (2.5%)
1	**Appearance**	8.00 ± 0.10^{a}	6.00 ± 0.20^{c}	7.00 ± 0.10^{b}	7.00 ± 0.10^{b}	7.00 ± 0.12^{b}
2	**Texture**	9.00 ± 0.05^{a}	6.00 ± 0.17^{c}	7.00 ± 0.12^{b}	7.00 ± 0.10^{b}	7.00 ± 0.15^{b}
3	**Color**	7.00 ± 0.10^{ab}	7.00 ± 0.15^{ab}	6.00 ± 0.12^{b}	7.00 ± 0.10^{ab}	7.00 ± 0.10^{ab}
4	**Flavour**	7.00 ± 0.10^{ab}	7.00 ± 0.10^{ab}	6.00 ± 0.10^{b}	7.00 ± 0.10^{ab}	7.00 ± 0.10^{ab}
5	**Aftertaste**	8.00 ± 0.10^{a}	6.00 ± 0.15^{c}	7.00 ± 0.10^{b}	6.00 ± 0.12^{c}	7.00 ± 0.10^{b}
6	**Overall Acceptability**	9.00 ± 0.08^{a}	6.00 ± 0.12^{c}	7.00 ± 0.10^{b}	7.00 ± 0.10^{b}	7.00 ± 0.12^{b}

Data are presented as mean ± standard deviation (n = 3). Different superscript letters within a column denote significant differences at $p < 0.05$ according to Duncan's multiple

4.0. CONCLUSION

The current research paper has displayed how protein-enriched cookies could be developed successfully through a partial replacement of refined wheat flour with modified flaxseed protein isolates. The pre-extraction de-mucilaging of flaxseed followed by extraction was much more effective to get a higher yield of proteins and the pre-treatment of flaxseed with microwaves was much more effective to make the isolate functional enough to be incorporated into bakery formulations. Out of the several formulations that were formulated cookies made of a 2% mixture of modified flaxseed protein isolate (F3) had better sensory acceptability, balanced texture, and good color properties compared to the other variants. Proximate analysis proved that the protein content in the flaxeed protein is progressively increasing with a higher incorporation level, which is an indication of the nutritional capability of flaxed protein as a fortifying agent. The results confirm that the altered flaxseed protein isolate may be effectively used as a component in the preparation of the nutritionally-enriched, plant-based cookies without the negative effect on the consumer perception. This will not only enrich the traditional bakery products in terms of functionality and nutrition but it will also offer a sustainable channel of utilization of the flax seed meal which is a useful by-product of the oil extraction sector.

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