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Formulation, Overall Acceptability, Nutritive Value Assessment of Gluten-free, Vegan Bread and Comparison with Contemporary Gluten-free Bread

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Authors' contributions

This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

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ABSTRACT

Gluten-free and vegan bakery items have been gaining popularity. Their demand has increased over the past few years, with India being one of the largest gluten-free food consumers. The gluten-free pieces of bread available in the market are crumbly in nature and have a dry mouthfeel, reducing its overall acceptability among consumers. Therefore, the present study was undertaken to develop gluten-free and vegan bread and enrich it with a functional component such as flaxseeds and assess its overall acceptability and nutritional value compared to gluten-free bread available in the market. Once the product was standardized for its serving size and yield, various sensory evaluation trials were conducted using 9 points hedonic scale on bread attributes with the help of semi-trained(n=30)and trained panellists(n=3). Proximate analysis of macronutrients was conducted along with the microbial analysis. The proximate analysis showed that the experimental product had a higher protein content(18.26g)and a lower fat content (5.58g) as compared to the market-bought control sample of gluten-free bread (Total protein content was 3.44g and total fat content was 9.20g). The experimental bread microbial analysis indicated that the total plate count,

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yeast and mold count, and coli form count were in the acceptable range. Salmonella and E.coli were absent in the sample Statistical analysis using paired t-test was performed found a significant difference between the experimental and control group among the following characteristics: taste/flavour, texture/mouth feel, and overall acceptability ($p \leq 0.05$). A shelf-life study of the bread was conducted, and it was seen that the bread remained sensorial acceptable for three days at a refrigerated temperature and four days at room temperature. The bread had a higher nutritional profile than the control sample and received higher overall acceptability.

Keywords: Gluten-free bread; vegan bread; Celiac disease; gluten-free bakery; high protein bread.

1. INTRODUCTION

The bakery sector is a fast-growing commercial sector and has seen an increase in demand for various healthy bakery items in the past few years. The bread market was valued to be about 82% in the bakery market as of 2016. In recent years, various health trends such as gluten-free foods, vegan foods, and many others have come into play in this sector. Several companies have started manufacturing vegan and gluten-free baked products to meet consumer demand. Among the gluten-free foods, the bakery and confectionary sectors hold the highest share. In India, the demand and consumption are also high due to individuals being at a high risk of celiac disease. Vegan diets are also followed in India. Vegan product manufacturing and sales in Southeast Asia have increased since 2012.

Bakery products are consumed extensively by all age groups in India due to their taste and easily digestible nature. A shift has been seen towards the intake of bakery items due to Western culture's increasing influence. Also, the consumers' rising health awareness has encouraged the manufacturers to focus on developing multi-grain, brown, and whole wheat bread. As bakery products provide both convenience and affordability to the consumers, their consumption level has increased in the past few years.

The bakery market in India has currently valued at Rs. 3295 crores where the bread holds about 82% share, as in 2016 [1]. India's total bread production is estimated to be around 3.75 million tones every year: South India consuming about 32% of total bread production in the country, followed by the north, which consumes about 27%, the west 23%, and the east 18%. Also, healthy bread variants have been gaining popularity [1].

In recent years, various trends such as vegan and gluten-free diets have increased, and its consumer base is increasing. The bakery and confectionary industry have started incorporating foods that do not contain gluten and plant-based foods. The growth of these food products in this sector has provided consumers with a wider variety of gluten-free and celiac foods. Products such as vegan ice creams and desserts, vegan bread, gluten-free snacks, and bread and pastries have gained popularity [2].

The gluten-free market in India has grown at a CAGR of 8.1% from 2013 to 2018. India is one of the largest consumers of gluten-free foods. Among the gluten-free foods, the bakery and confectionary items have been more in demand, and these two sectors hold the largest segment in the gluten-free foods market [3]. The demand for gluten-free products in India increased as wheat is a staple diet in northern India, and this led to a higher risk of celiac disease among the consumers [4]. The prevalence of celiac disease is increasingly becoming prevalent in India, in the northern region, due to higher wheat consumption. The haplo types involved in CD are more prevalent in this country's belt [5]. Celiac disease occurs due to the ingestion of gluten-containing products, which cause an allergic reaction leading to intestinal and extra intestinal symptoms [6].In, there are various classifications and CD symptoms, the most common symptoms being- diarrhea, anemia, abdominal pain, bloating, and constipation [7]. The, only treatment for individuals suffering from this condition is consuming completely free foods from gluten, such as quinoa, teff, buckwheat, amaranth, and excluding gluten-containing foods such as wheat, barley, and rye. However, a continuation of this treatment can lead to several dietary deficiencies such as foliate, zinc, fiber and can increase the proportion of ingestion of saturated fats, carbohydrates, sugar, and sodium, leading to metabolic syndrome. Also, these products have a high GI [8].

In [9], Gluten-free products need a binding agent as gluten is absent, and hence a network cannot

be formed. Hence, a mixture of several flours should be used rather than single flour [10]. Also, these products are crumbly in nature and have a dry mouth feel [11]. Legume flours help to provide structure to these products along with improving the volume of products. Pseudo-cereals help to delay staling of gluten-free products and to reduce hardness. Starchy flours along with starch help in providing a binding network to act as gluten [12]. Gluten-free products tend to be harder than normal bread and less springy. Hence the overall acceptability of gluten-free bread is low.

Another latest trend in healthy diets is the Vegan Diet. Veganism started due to factors such as health improvement, ethical issues, and religious motivations. In 2018, UK launched the most vegan products, and almost half of the UK vegans started the following veganism in 2018. The number of vegans has increased exponentially from 2014 onwards. Apart from the UK, Australia became a popular country following vegan diets in 2018, followed by New Zealand. In the USA, the number of vegans has increased from 4 million to nearly 19 million in 2017. Germany became the global leader in launching vegan products in 2018, while Sweden reduced meat consumption by about 2.6% in 2017. Southeast Asia saw an increase in product launches of vegan foods between 2012 and 2016 [13].

1.1 Aims and Objectives

The objectives of the present study were as follows:

- To standardize the gluten-free, vegan bread
- To assess the sensory acceptability of the product
- To assess the nutritional value of the formulated bread.
- To assess the shelf life of the formulated bread
- To compare the overall acceptability and nutritional value of the product with market available gluten-free bread

2. MATERIALS AND METHODS

2.1 RAC Approval

Approval for conducting the study was taken from the Research Advisory Committee of Symbiosis School of Biological Sciences, Pune. An informed consent form was taken from the panel members before the initiation of food product development.

2.2 Study Locale

Nutrition Laboratory, Symbiosis School of Biological Sciences, Symbiosis International University, Pune, India

2.3 Preparations

A review of the literature was done to obtain scientific data on formulating gluten-free bread. Data were collected on various formulations existing and the function of ingredients involved in gluten-free baking.

2.4 Procurement of Raw Ingredients

Chickpea flour, buckwheat flour, arrowroot flour, baker's yeast, table salt, granulated sugar, rice bran oil, and raw flaxseeds were procured from local markets in Pune city keeping in mind the acceptability of these ingredients by gluten-free and vegan consumers. All ingredients were purchased after analysing their economic cost and their overall acceptability.

2.5 Pre-Standardization

Various trials were conducted to achieve a product with desirable acceptability. Different proportions of buckwheat flour, amaranth flour, and chickpea flour were used in different trials, and its overall acceptance was noted.

2.6 Standardization

The standardized recipe was formulated after conducting various trials. The yield of the product and serving size of the bread was calculated. Also, moisture loss during baking was calculated.

Standardization of a recipe helps produce the same yield and taste whenever the recipe is prepared with the same procedure, equipment, and quantity of ingredients used. It helps to avoid wastage and helps to provide a consistent nutritional value per serving every time.

The standardized recipe ingredients were chickpea flour, buckwheat flour, arrowroot flour, fresh yeast, granulated sugar, table salt, rice bran oil, flaxseeds, and water (ice cold). The

types of equipment used measured bowls and spoons, fork, measuring cylinder, aluminium bread tin, spatula, whisker, cutting knife, cooling rack, and hand gloves. Other equipment's which were used were analytical balance, proofer, and commercial baking oven. The results are mentioned in Table 1 and Fig. 1.

2.7 The Rationale for Selecting Ingredients

The flours were selected based on their functional properties in gluten-free baking. Chickpea flour is high protein flour, was used, buckwheat was chosen as it is a good source of fibre, fibre mixture group--fed 100 g of fibre mixture/kg of diet (soya polysaccharide, insulin, resistant starch, Arabic gum, fructo-oligossaccharide and cellulose) and arrowroot flour was chosen as it is light in texture and a starchy flour helps in binding. Flaxseeds were incorporated as a functional ingredient and as a substitute for chemical binders used in gluten-free products. Fresh yeast was chosen as it provides a greater rise during proofing required for bread than active dry yeast. In [14], sugar was used to improve fermentation, and salt was used to provide controlled fermentation. Oil was used as a preservative to improve the shelf life. Calcium propionate was considered as a preservative to improve the shelf life of the bread. It does not have any effect on yeast fermentation and is hence used.

2.8 Panel Selection

For the selection of panels, a sensory test was conducted among 30 individuals selected on a random basis. They were provided with six salt solutions of concentrations 1%, 1.5%, 2.5%, 3%, 3.5%, and 4.5% and were asked to rate these solutions as the saltiest and lowest salt solutions. Nineteen panellists among these individuals could identify these solutions correctly and hence were chosen for sensory evaluation as semi-trained panellists.

Fig. 1. Pictorial presentation of informal trials

Trained panellists were chosen as Symbiosis School of Culinary Arts students and two chefs from the same institute.

2.9 Sensory Evaluation

Sensory evaluation was conducted in a well-lit testing area with a comfortable seating area and drinking water facilities.

Different methods were used for sensory evaluation for the semi-trained panellists. The first method used was the paired comparison test, where semi-trained panellists must identify parameters such as taste, color, texture, and odor of the experimental sample, i.e., the product prepared and control sample, i.e., purchased from the supermarket. The second method used was the hedonic rating test. The panelist is asked to find the samples' acceptability on a seven or a nine-point scale from dislike extremely to like extremely. This test consisted of an evaluation form which consisted of characteristics such as appearance/color, texture, flavor, odor, and overall acceptability. The third method was the flavor profile test, where the aroma, taste, and mouth feel of the product had to be described in qualitative and quantitative terms.

For the trained panelists, the sensory evaluation of various aspects of the product, such as crust characteristics, crumb color, texture, flavor, and chewing characteristics of the product, had to be described. The results are mentioned in Figs. 3 and 4.

2.10 Proximate Analysis

Proximate analysis and microbial analysis were conducted at an ISO Certified and NABL Accredited Laboratory: Anushka Food and Water Testing Laboratory, Pune.

The analysis was performed by the methods such as estimation of proteins by Kjeldahl Method, estimation of total fat by Soxhlet Extraction Method, estimation of reducing sugar, invert sugar and total sugar by Lane and Eynon Method, estimation of moisture by a hot air oven, and estimation of ash by a muffle furnace. Estimation of carbohydrates and energy from food was done by using formulas for calculation. The results are mentioned in Table 2.

2.11 Microbial Analysis

The analysis included the qualitative determination of total plate count by pour plate method, coli form by pour plate method, enumeration of yeast and mould by pour plate method, and isolation and identification of *staphylococcus aurous*. **Staphylococcus aureus** is the most dangerous of all of the many common **staphylococcal** bacteria that often cause skin infections but can cause pneumonia, heart valve infections, and bone infections.

The results are mentioned in Table 4

2.12 Nutritional Value Information

The experimental sample's nutritional information was calculated by the use of information on individual ingredients provided by the National Institute of Nutrition (NIN).

2.13 Statistical Analysis

Numerical data obtained from the sensory evaluation by trained and semi-trained panellists were tabulated into the Excel Sheet. It was then used in the SPSS software to calculate the overall acceptability of each characteristic of the experimental product compared to the sample product with the use of paired t-test. The statistical tests used were the Independent t-test and Inter reliability test. The results are mentioned in Table 5

2.14 Cost Estimation

The cost of a food product includes consideration of various costs during the preparation of the product. It includes the product pricing, cost of raw materials, and cost of utilities and energy costs. Labor costs are also included in these costs [14].

The total cost, which was calculated, included the cost of raw materials and electricity, fuel, and labor costs. The total amount for one serving was calculated based on the price of ingredients procured from the local market. The total cost of the product was calculated based on all direct and indirect costs.

2.15 Shelf Life Study

The shelf life of food is the period throughout which the food product retains its organoleptic properties and is acceptable in terms of safety point of view. Shelf-life studies help provides information to both the manufacturers and consumers to ensure that the product retains its quality during storage [15].

The product's shelf-life study was conducted by preparing two samples of the bread and then storing it in a cling wrap after cooling down

completely. The samples were kept at room temperature and in a refrigerator, respectively. Both the samples were checked for their change in taste, color, texture, odor, or appearance by sensory evaluating at an interval of one day. The number of days for which the bread did not spoil was noted. The observations were noted, and the shelf life was determined.

2.16 Packaging and Label Designing

The packaging is done to preserve and protect the food product and ensure the convenient and safe distribution of food products. It is important to choose packaging which would not leach into the food product and make it unsafe for human consumption. The finished product packaging was done in parchment paper, which was packaged in a hard paper box. It was stored at room temperature. Parchment paper helps to provide mechanical strength and is biodegradable [16]. It is impervious to oil and water; however, it is not heating sealable [17]. Where, Fig. 2 discussed about Standardised trial.

3. RESULTS

3.1 Standardization

Table 1. Ingredients of the standardised trial

Sl.no	Ingredients	Amount (GMS)
1	Arrowroot flour	60g
2	Buckwheat flour	37g
4	Chickpea flour	95g
6	Fresh yeast	7g
7	Salt	3g
8	Water (Ice cold)	200ml
9	Sugar	12g
10	Oil	10g
11	Flaxseed powder	15g

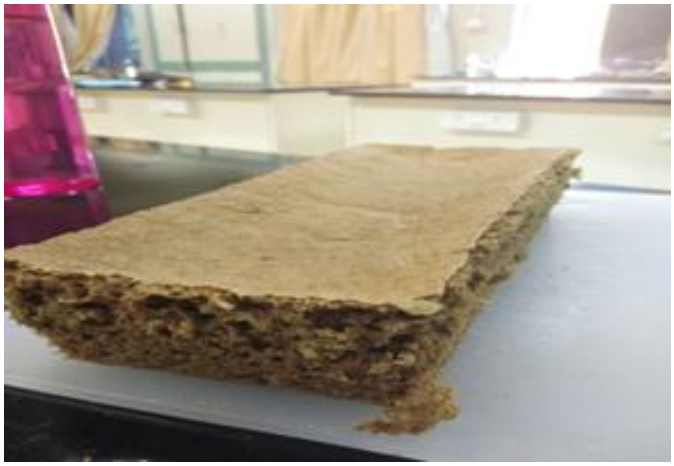

Fig. 2. Standardised trial

3.2 Product Yield

Weight of uncooked batter (weight before baking) = 299g
Weight of the final product (weight after baking) = 250g
Number of slices: 8
Weight of one slice: 30g
Weight of one serving: 30g

3.3 Sensory Evaluation

The following table compares the mean scores of the hedonic rating between the experimental and control sample by semi-trained panellists. The y-axis indicates the parameters of sensory evaluation, while the x-axis indicates the scores obtained. The scores for all parameters were higher for the experimental sample as compared to the control sample.

A comparison was done between both the samples among the semi-trained panelists for its overall acceptability. It was found that 56% of the panelists considered the experimental sample as more acceptable. The trained panellists evaluated various characteristics of the experimental sample. The crust color was considered to be both dark brown and golden brown among the panelists. Most of the panelists found the crust to be intermediate rather than thin and thick. The crumb color was evaluated as brown by all panellists. The texture was considered to be slightly open by most of them. The flavor and taste were rated as pleasant, while the chewing aspect was chosen as both chewy and weak majorly.

3.4 Nutritional Analysis

A comparison between the control and experimental sample (results of the NABL accredited laboratory's proximate analysis) is given details below in Fig. 5.

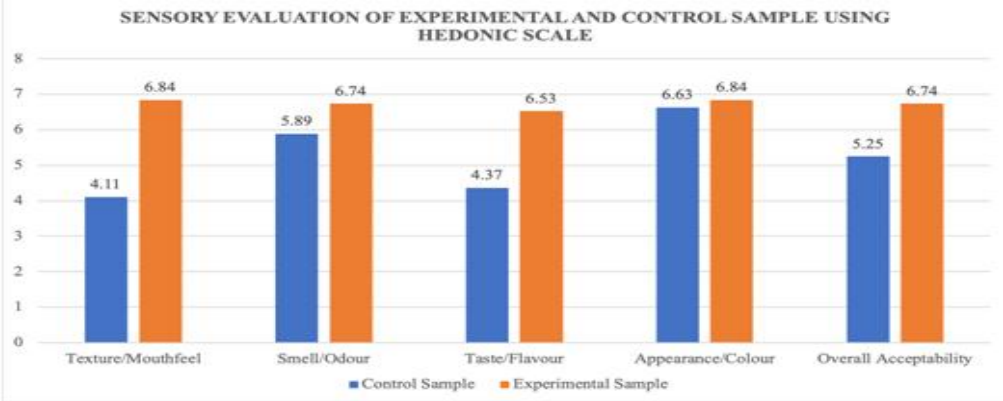

Fig. 3. Sensory evaluation of experimental and control sample using hedonic scale

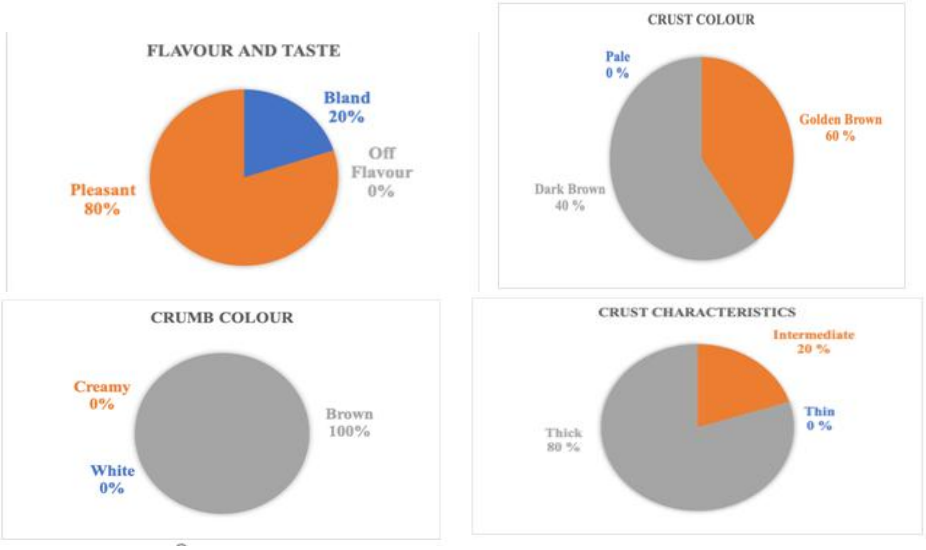

Fig. 4. Comparison between characteristics of experimental sample

Table 2. Comparison of nutritional value between control sample & experimental sample (per 100 gm)

Sl.no	Parameters	Result (Control sample)	Result (Experimental sample)
1	Energy	311.56 kcal	274.98kcal
2	Carbohydrate	53.75g	37.93g
3	Proteins	3.44 g	18.26g
4	Fats	9.20g	5.58g
	MUFA	2.66g	-
	PUFA	4.32g	-
5	Total Sugars	Not Detected	3.01g
6	Moisture	-	36.39g
7	Total Ash	-	1.84g

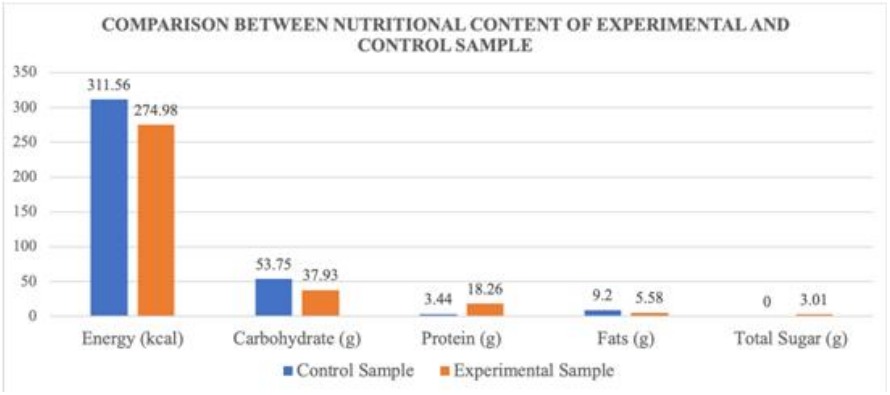

Fig. 5. Comparison between nutritional content of experimental and control sample

The % of Daily Value for 2000 kcal for the macronutrients was calculated and is as follows in Table 3.

Table 3. % Daily value (2000kcal diet)

Nutrients	% DV	DV (USDA)
Energy (kcal)	13.7	2000
Proteins (gm)	36.5	50
Fats (gm)	8.5	65

3.5 Microbial Analysis

The microbial analysis was conducted at an ISO and NABL certified laboratory. The experimental bread microbial analysis indicated that the total plate count, yeast and mould count, and coli form count were in the acceptable range from day zero to day four. *Salmonella* and *E.coli* were absent in the sample till day four.

Table 4. Microbial parameters

Sl.no	Parameters	Results	Limits
1	Total Plate Count	3 x 102cfu/g	1 x 103 cfu/g
2	Coli forms	<10 cfu/g	20 cfu/g
3	Yeast and mould	4 cfu/g	10 cfu/g
4	E.coli	Absent (per g)	Absent (per g)
5	Salmonella	Absent (per 25g)	Absent (per 25g)

Table 5. Mean comparison of experimental and control samples

Group statistics					
	Groping	**N**	**Mean**	**Std. Deviation**	**Std. Error Mean**
Appearance	E	19	6.84	1.259	0.289
	C	19	6.63	0.895	0.205
Taste	E	19	6.53	1.073	0.246
	C	19	4.37	0.761	0.175
Odor	E	19	6.74	1.046	0.240
	C	19	5.89	0.737	0.169
Texture	E	19	6.84	1.214	0.279
	C	19	4.11	1.100	0.252
Overall acceptability	E	19	6.74	0.922	0.212
	C	19	5.25	0.382	0.088

3.6 Statistical Analysis

A significant difference between the experimental and control groups were the following characteristics: Taste/flavor, Texture/mouth feel, and overall acceptability using paired t-test. The mean of the overall acceptability of experimental and control groups were 6.74±0.922 and 5.25±0.382, respectively.

An independent sample t-test indicated a significant difference ($p<0.05$) in the taste, smell, texture, and mouth feel with 0.000, 0.007, 0.000, and 0.000, respectively. The characteristic of appearance did not show to have a significant value.

3.7 Shelf life Study

The sample kept in the refrigerator remained unstable for up to 3 days. The sample kept at room temperature remained unstable for up to 4 days.

3.8 Cost Estimation

The cost per ingredient was calculated, and the total cost of the bread loaf ingredients was calculated to be Rs 2.19. The total serving of bread was eight slices, and the cost was calculated to be Rs 17.5. Other Miscellaneous costs considered were energy cost as Rs 0.7, labor cost considered as nil as researchers at the lab developed the project. Packaging cost at Rs 7 and other production costs at Rs 0.7. The final cost of the product was calculated to be Rs 26. The cost of the control sample was Rs 120.

4. DISCUSSION

The present study focuses on developing a vegan and gluten-free bread enriched with flaxseeds as a functional component. The addition of flaxseeds helped to improve the batter workability as flaxseeds have a high-water binding capacity. It also helped in improving the crumb hardness and the loaf volume of the bread. The addition of flax seeds helped improve the nutritional content as it is high in dietary fiber. Various trials were conducted on developing bread with an acceptable consumer rating. Several different proportions of ingredients were changed, and even the method of preparation was changed. This is due to the fact that gluten-free products do not contain gluten as a binding agent, and hence it is a challenge to replicate the viscoelastic properties of gluten. A major challenge was to obtain bread with a good binding network and rise. After the bread's final trial was developed, the moisture loss from bread was calculated by subtracting the final weight of the bread from the initial batter weight. The total serving of bread along with the weight of a serving was estimated. The sensory evaluation conducted among semi-trained panellists indicated that the experimental sample had greater acceptability in flavor, texture, color, mouth feel, and overall acceptability. The experimental sample's acceptability was higher as it did not have a dry and chewy taste similar to the control sample and had an overall pleasant taste. The mean average scores of all aspects were found to be higher in the experimental sample as compared to the control sample. The comparison between the experimental sample's overall acceptability and the control sample showed that the experimental sample had 56% of overall acceptability. The trained panellists evaluated the bread on aspects such as crust color, crumb color, crust characteristics, flavor and taste, texture, and chewing parameters. It was found that crust color was considered as dark brown by about 60% of the panellists, the crumb color was chosen as brown by all the panellists, the crumb characteristics were rated

as intermediate by 80% of the panellists, the flavor was considered to be pleasant by 80% of the panellists and the texture of the bread was chosen as slightly open by 80% of the panellists. The overall acceptability of the bread was considered to be good. The preference test, which the semi-trained panellists filled, showed that aspects such as taste, texture, and the overall mouthfeel were more acceptable for the experimental sample. The odor for both the samples was likable. The comments indicated that the control sample's texture was extremely dry and chewy, and the taste was raw and unappealing. The texture of the experimental sample was better due to the addition of flours such as chickpea (chickpea is high in protein which helps in mimicking the properties of gluten and improving the softness of loaf), buckwheat (which helps in reducing the crumb hardness), and arrowroot (arrowroot is high in starch; starch along with proteins help in improving the batter consistency). A comparison between the experimental and control sample's nutritional value showed that the experimental sample was higher in proteins and sugar and was lower in energy, fats, and carbohydrates compared to the control sample. The difference in means of the control and the experimental sample was greater in taste, smell, texture, and overall acceptability.

The statistical test conducted (Paired sample t-test test) indicated that there was a significant difference in taste, smell, texture, and mouth feel. ($p<0.05$).The experimental bread microbial analysis indicated that the total plate count, yeast and mould count, and coli form count were in the acceptable range. *Salmonella* (**Staphylococcus aureus** is the most dangerous of all of the many common **staphylococcal** bacteria that often cause skin infections but can cause pneumonia, heart valve infections, and bone infections.) and *E.coli* were absent in the sample till day four. The shelf-life study indicated that the bread is safe to consume within four days of manufacturing when kept a room temperature and three days of manufacturing when kept at a refrigerated temperature. The total product cost was calculated to be Rs 26 compared to the market cost of the gluten-free bread, which was Rs 120. The materials used in the experimental product were locally available, and no artificial colors or additives were used, which made the product more cost-competitive.

5. CONCLUSION

The experimental product had higher acceptability and nutritional value as compared to the experimental sample. It was vegan, gluten-free, and the bread was high in protein and fiber and enriched with flaxseeds. This gluten-free bread is a nutritious alternative and is more economical as compared to the existing gluten-free slices of bread available in the market and hence has good scalability for commercial bakeries. The formulated gluten-free, vegan bread is a healthier, protein-rich alternative added in the range of products to contemporary vegan breads available in the market, suitable to gluten intolerants, celiac disease patients, and lactose intolerant. This proposed method's advantage is the product adds to a range of healthy vegan, gluten-free range of products. Semi-trained and trained panellists were included for sensory evaluation and product improvement to check commercial acceptability. Proximate analysis and microbial analysis were certified by NABL accredited laboratory. This paper's scope and recommendation is the product has a strong potential to be served in vegan restaurants with variations using other gluten-free, vegan options. The product may be subjected to consumer research to check for commercial acceptability.

CONSENT

It's not applicable.

ETHICAL APPROVAL

Not applicable as the research was a food product development laboratory-based research.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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