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Preparation and shelf-life evaluation of Flaxseed incorporated biscuits

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Abstract

Flaxseed incorporated biscuit is a baked, unleavened cake that uses flaxseed flour as the main component. The development of germinated flaxseed incorporated biscuit and the study of its storage stability were the main objectives of this study. Flaxseed was germinated up to 7 days at room temperature. Germination longer than 7 days resulted in significant mold growth. For this study, flaxseed flour was made from the 7- day germinated flaxseed. Five formulations were tasted in a laboratory setting using 0, 12.5, 25, 37.5, and 50 parts germinated flaxseed flour to wheat flour. Design Expert v13 software was utilized to formulate the recipe using D-optimal design. One way ANOVA without blocking was carried out at a 5% level of significance to measure consumer acceptability. According to mean sensory scores, a formulation containing 25 parts flaxseed flour was chosen for further analysis. Acid, peroxide, and moisture values were used to determine the shelf-life of the formulated biscuits. At the end of two months, the values obtained —0.21 mg KOH/g oil, 1.8 MeqO2/kg fat, and 4.88% were within the safer limits. Overall, flaxseed flour incorporation of 25 parts of flaxseed flour was successfully incorporated into the biscuit recipe. Calcium, iron, protein, ash, fiber, and fat were higher in the optimized product than the control.

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1. Introduction

The baking industry is one of the leading systematized food industries. A number of reasons contribute to the popularity of bakery products, including their convenience, low cost, ready-to-eat nature, ease of transportation, and variety in taste and texture. Biscuits are very popular ready-to-eat foods made of flour, fat, sugar, water, milk, salt, and chemical yeast as raising agent (Manley, 2000). Cereals, millets, and other ingredients can be added to bakery products to enhance their nutritional value. It is therefore possible to deliver functional ingredients to consumers through these products (Tiwari & Mishra, 2019).

Due to market competition and growing consumer demand for healthy, natural, and useful products, efforts are being made to adjust the nutritional composition of biscuits to raise their nutritive value and functionality. It is common to achieve these effects by increasing the ratio of raw materials other than wheat or dietary fiber in basic recipes, in order to increase the biscuit's protein and mineral content for quality and availability or to increase dietary fiber content and improve prebiotic characteristics. They are widely accepted and consumed by almost all profiles of consumers from many countries as they serve as an important source of nutrients. However, several studies indicated that the nutritional, physical, and sensory characteristics of biscuit depend on both the physiochemical properties of the flour and the processing method employed for flour preparation (Asif-Ul-Alam et al., 2014).

The annual herb flax (Linum usitatissimum), a member of the Linaceae family, has blue flowers and produces small, flat seeds that range in color from golden yellow to reddish brown. Flaxseed contains a crunchy surface and nutty flavor (Rubilar et al., 2010). Flaxseed is also known as linseed, and the two terms are frequently used interchangeably. The benefits of flaxseed include oil rich in omega-3, soluble fibers, lignans, vitamin E, proteins, and carbohydrates that meet the basic needs of the human diet and maintain health (Bernacchia et al., 2014; Ganorkar & Jain, 2013). Lignans, flavonoids, and phenolic acids are among the other bioactive components of flaxseed that belong to the phenolic compound class.

In addition to its nutritional and therapeutic benefits, flaxseed naturally contains anti-nutritional elements such as cyanate, oxalate, phytic acid, and tannin. These anti-nutritional substances prevent the correct absorption, digestion, and utilization of nutrients (Goyal et al., 2014). It must be prepared and stored to preserve as much of its medicinal and nutritional value as possible and to minimize anti-nutritional elements. One of the best processing techniques for boosting the nutritional value of pulses and legumes is germination, which increases the digestion of elements like protein and carbohydrates (Kumar et al., 2021).

The use of flaxseed in food items will help protect against cancer, and lower cholesterol levels, diabetes, and heart disease. Flaxseed is one of the main sources of phytochemicals (Gutte et al., 2015).

Flaxseed (Linum usitatissimum) also known as linseed is incorporated into various baked and other products such as muffins (Ramcharitar et al., 2005; Sudha et al., 2010), bread (Marpalle et al., 2014), biscuits (Masoodi & Bashir, 2012), bagels (Aliani et al., 2012), cake (Trevisan & Arêas, 2012), energy bar (Mridula et al., 2013) and cereal bars (Khouryieh & Aramouni, 2013).

Research is needed to create new goods that not only incorporate nutritional and functional characteristics but also take consumer acceptance into account. This is due to the increased consumer demand for food that has both nutritional and sensory quality as well as functional claims. High consumer acceptance makes bakery goods like biscuits crucial for introducing bioactive substances into the human diet (Rathi & Mogra, 2012). The objectives of this study were to incorporate germinated flaxseed flour in the wheat flour to prepare flaxseed flour incorporated biscuits, assess the sensory attributes and cost of the optimized product, and study their storage stability and consumer acceptance.

2. Materials and Method

2.1. Primary components

Refined wheat flour used for biscuit making was obtained from the local market of Dharan. Flaxseed was bought from Birtamode, Jhapa. The other raw materials such as sugar, salt, and fat in the form of butter (Amul, India), skimmed milk powder, and baking powder was bought from the local market of Dharan. Lecithin was kindly provided by Asian Thai Foods, (Sonapur, Sunsari). Sodium bicarbonate, ammonium bicarbonate, and flavor (vanilla) were obtained from the laboratory of Central Campus of Technology, Dharan.

The purity percent of raw materials were salt- 1.9%, amul butter- 80%, skimmed milk powder- 98%, baking powder- 99%, ammonium bicarbonate- 98.5%, sodium bicarbonate- (99-100) % & vanilla flavor- 13.35%

2.2. Chemicals required

The required chemicals obtained from the laboratory of Central Campus of Technology are mentioned below: Sodium hydroxide (NaOH), Hydrochloric Acid (HCl), and Sulphuric acid (H_2SO_4) were bought from Thermo Fisher Scientific India Pvt. Ltd. Boric acid (H_3BO_3), Oxalic acid ($C_2H_2O_4$) and Ammonium oxalate ($C_2H_8N_2O_4$) were bought from Merck (India) ltd. Phenolphthalein indicator ($C_{20}H_{14}O_4$) was bought from Merck life Pvt. Ltd. Potassium permanganate ($KMnO_4$) was bought from Avantor Performance Materials Ltd. Distilled water (H_2O) was bought from the biochemistry lab of Central Campus of Technology. Saturated potassium persulfate ($K_2S_2O_8$), Toulene (C_7H_8), and Copper sulfate pentahydrate for digestion mixture ($CuSo_4.5H_2O$) were bought from Qualigens fine chemicals. Neutral alcohol (C_2H_5OH) was bought from Bengal chemicals and pharmaceuticals Ltd. Bromocresol green ($C_{14}H_{14}N_3NaO_3S$) for mixed indicator solution was bought from Himedia laboratories Pvt. Ltd. Methyl orange indicator ($C_2H_8N_2O_4$) and Potassium sulfate for digestion mixture (K_2SO_4) was bought from Merck KgaA, Germany. Mohr's salt [$FeSO_4(NH_4)_2SO_4.6H_20$] was bought from Chem lab supplies. Potassium oxalate ($K_2C_2O_4$) was bought from Oxford lab fine chem LLP. Sodium thiosulfate ($Na_2S_2O_3$), Starch $(C_6H_{10}O_5)_n$, Acetic Acid (CH_3COOH), Acetone (C_3H_6O), Ammonia (NH_3), Silver nitrate (AgNO3), Potassium thiocyanide (KSCN), Petroleum ether (C_6H_{14}), Methyl red ($C_{15}H_{15}N_3O_2$) for mixed indicator solution, Selenium dioxide (SeO_2) for digestion mixture, Ammonium hydroxide (NH_4OH) and Potassium iodide (KI) were bought from Fisher Scientific India Pvt. Ltd.

2.3. Preparation of germinated flaxseed flour

2.3.1. Cleaning

Flaxseeds were first winnowed with woven bamboo trays (nanglo). In this step; husks, immature seeds, and light particles were winnowed away and heavier particles such as specks and stones were separated by gravity during winnowing.

2.3.2. Steeping

Cleaned seeds were transferred to a stainless-steel vessel and water was added at the rate of 1.5 times that of flaxseed weight. Light materials present in the seeds were skimmed off. Agitation was done to clean the seeds. The seeds were steeped for 24 h at room temperature of 21°C and finally drained to remove the excess water.

2.3.3. Germination and drying

To drain the extra water, the soaked seeds were first gathered in a muslin towel and swirled. The seeds were placed in a humidity chamber and incubated in an incubator for seven days at (23±3) °C with a RH of 90% to facilitate germination. For seven days every day, roughly 500 grams of the seeds were taken and dried in a cabinet dryer at (50±3) °C until the moisture content of the seeds reached 10%.

2.3.4. Grinding and packaging

The dried seeds from each day were ground into a powder, sieved, and then put into a high-density polythene bag (52 µm). Consequently, seven distinct samples were prepared for analysis.

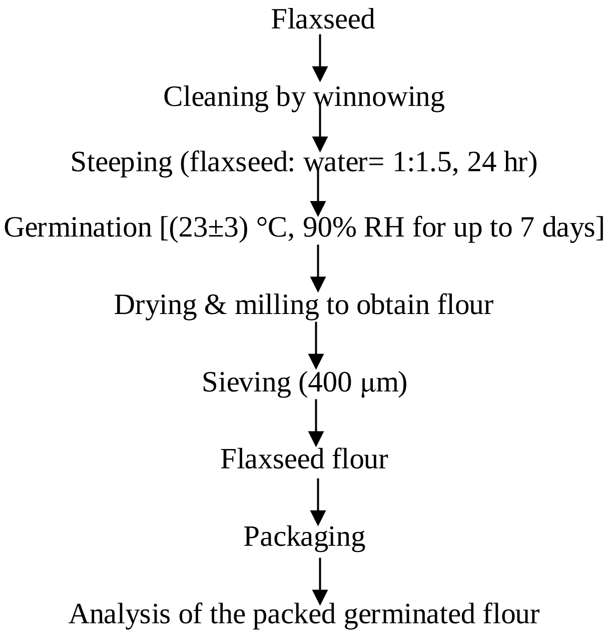


Fig 1. Preparation of germinated flaxseed flour

2.4. Determination of threshold of flaxseed flour

The determination of the threshold for flaxseed flour was carried out with the help of a trial experimental design (Table 1). The result of the experiment suggested that germinated flaxseed flour of more than 50% in the biscuit recipe did not result in acceptable biscuit quality. Therefore, the threshold for flaxseed flour was set between (-0 and-50-) %.

Table 1. The recipe was made with Design Expert v13 software (Stat-Ese Inc., United States). The recipe was created using D-optimal design.

Run	Component 1 A: Flaxseed flour	Component 2 B: Wheat flour
1	50	50
2	0	100
3	25	75
4	37.5	62.5
5	12.5	87.5

2.5. Formulation of recipe

The flaxseed flour-infused biscuit recipe was prepared according to the recipe in Table 2.

Table 2. Recipe formulation (A, B, C, D & E) of the flaxseed flour incorporated biscuit types.

Components	A	B	C	D	E
Wheat flour	100	87.5	75	62.5	50
Flaxseed flour	0	12.5	25	37.5	50

The fixed ingredients used are: 35 parts fat, 35 parts sugar, 0.3 parts salt, 6 parts skimmed milk, 1.5 parts baking soda, 1.5 parts baking powder, 1.5 parts NH_4HCO_3, 0.75 parts $NaHCO_3$ 1.5 parts lecithin, 0.1 parts Vanilla flavor and 10 parts Water. Source Poudel, 2011.

2.6. Formulation of biscuit recipe with incorporation of germinated flaxseed flour

Cream was first made by combining fat, sugar powder, and soy lecithin. Water and salt were dissolved and added to the cream mixture. Skimmed milk powder and ammonium bicarbonate were next added for the final creaming of the cream mixture. All of the flour and baking powder were added and thoroughly combined as the creaming process continued. The prepared dough was sheeted (5–6 mm) thick and was cut to form a circle shape. The formed biscuit sheets were baked for 15- 20 minutes at 160°C. The biscuits were packed in the HDPE packets (52 μm) after cooling to about 35°C.

Blending butter

↓

Addition of baking powder and soda

↓

Addition of skimmed milk powder, Ammonium bicarbonate

↓

Mixing

↓

Addition of flour and mixing

↓

Kneading

↓

Rolling and cutting

↓

Baking (160°C, 15-20 mins)

↓

Cooling (at room temperature)

↓

Packaging

↓

Storage (at room temperature)

Fig 2. Flow chart for the preparation of flaxseed flour incorporated biscuit adopted from Lengure et al. (2018)

2.7. Evaluation of the product

2.7.1 Analysis of flaxseed flour incorporated biscuits and wheat biscuits

Spread Ratio

Using the formula as per AOAC (2005), the biscuit's spread ratio was calculated.

$$\text{Spread ratio} = \frac{\text{Diameter (mm)}}{\text{Thickness (mm)}}$$

Vernier calliper was used to measure the diameter and screw gauge was used to measure thickness.

Volume

According to the formula as per AOAC (2005), the volume of the biscuit was calculated by multiplying its area by its thickness.

$$\text{Volume (cm}^{3)} = \frac{\pi d^2 t}{4}$$

Where, t = Average biscuit thickness (mm)
d = Biscuit diameter (mm)

Density

The mass-to-volume ratio of the biscuit was used to determine the biscuit's density per AOAC (2005)

$$\text{Density (g/cm}^3) = \frac{\text{Mass (g)}}{\text{Volume (cm}^3)}$$

2.7.2. Physicochemical analysis

Moisture content

Moisture content of the flour and biscuit samples were determined by the weight loss determination by heating in a thermostatically controlled oven at 105°C for 1 hr (KC & Rai, 2007).

Ash content

In a Muffle furnace, flour and biscuit samples were heated to between 550 and 600 degrees celsius until a white, gray ash formed (KC & Rai, 2007).

Crude fat

The amount of crude fat in samples of flour and biscuits was measured using the Soxhlet extraction method and a Soxhlet apparatus with petroleum ether. Syphoning was used to repeatedly remove fat (KC & Rai, 2007).

Crude fiber

The crude fiber content of flour and biscuit samples was determined by using Buchner's filtration assembly (KC & Rai, 2007).

Crude protein

By using an automatic digestion and distillation system, the Kjeldahl method was used to determine the protein content of wheat and biscuit samples. The nitrogen content was used to compute the protein value (N2). For flour and biscuit samples, a factor of 6.25 had to be applied (KC & Rai, 2007).

Carbohydrate

The percentage of carbohydrate content was calculated using the difference between 100 and the total of the contents of moisture, protein, crude fat, fiber, and ash (KC & Rai, 2007).

Iron content

After digesting flour and biscuit samples with sulphuric acid, the resulting aliquots were utilized to measure the iron content in a spectrophotometer at 480 nm (KC & Rai, 2007).

Calcium content

Aliquots prepared for the determination of iron content were utilized to measure the calcium content by titration with standard potassium permanganate (KC & Rai, 2007).

Energy value

The control sample and the biscuit sample both had a calorific value of 4 × protein + 4 × CHO + 9 × fat = energy (kcal).

Determination of phytic acid

Trichloroacetic acid was used to extract the phytic acid, which precipitated as ferrous salt. Colorimetric analysis was used to evaluate the precipitate's iron content, and phytate phosphorus content was derived from that result using the assumption that the precipitate's 4Fe:6P molecular ratio was constant (Sadasivam & Manickam, 2016). The result was given as mg of phytic acid per 100 g of sample.

$$\text{Phytic acid (mg/ 100 g) sample} = \frac{\mu\text{gFe} \times 15}{\text{wt of sample (g)}}$$

Determination of tannin

The Folin-Dennis method was used to determine the tannins. Tannin-like substances in an alkaline solution react with phosphotungstomolybdic acid to form a brightly colored blue solution, the intensity of which was inversely correlated with the concentration of tannins. A spectrophotometer was used to measure the intensity at 760 nm (Sadasivam & Manickam, 2016).

2.8 *Sensory analysis*

Twelve semi-trained panelists participated in the sensory assessment for overall quality (teachers and students of Central Campus of Technology). Color, flavor, crispiness, texture, and general acceptance were the criteria for sensory evaluation. A hedonic rating exam was used to assess the senses (Lim et al., 2009).

2.9 *Statistical analysis*

Gene stat version 12.1.0.3338 statistical software was used to statistically evaluate the data. The Fisher's Protected LSD method was used to compare the data's means at the 5% level of significance.

2.10 *Packaging and storage of the biscuit*

The biscuits were packaged in a high-density polyethylene bag (52 µm) and kept at room temperature.

2.11 Determination of shelf life

The shelf-life of the product in the package under specified conditions can be confirmed by several methods, viz., (1) weight gain or loss method, (2) method based on testing the performance of the product, (3) chemical (acid value, peroxide value) changes during storage. All these tests are related to water vapor and oxygen permeability of the packaging material, which in turn indicates the increase of acid value and peroxide value of the product over time. All shelf-life assessment methods use accelerated and controlled conditions so that an accurate prediction of shelf-life can be possible within a short time (Kumar, 2001).

The acid value of more than 0.3 mg/KOH gm oil, peroxide value of more than 3 meq O2/kg fat, and moisture content of more than 6% till the last day of analysis indicates the end of the shelf-life of the product (Hooda & Jood, 2005).

To determine the biscuits' shelf life, the acid value, peroxide value, and moisture content of the biscuits were assessed.

Acid value

To extract free fatty acids for acid value, 5 ml of oil was combined with neutralized rectified spirit. The amount of the latter is then determined by titrating with standard NaOH or KOH while utilizing the phenolphthalein indicator. The mixture may be heated to around 70°C and vigorously stirred to aid extraction (KC & Rai, 2007).

Peroxide value

5 ml of a sample of oil was weighed in an iodine flask to which 25 ml of the solvent was added and CO2 was displaced. 1ml of KI solution was added, which was then left there for 1min (with gentle shaking). A few drops of the starch indicator were introduced along with 35ml of distilled water. Iodine released was titrated with 0.01N or 0.1N sod-thiosulfate until the blue tint just started to fade. Simultaneously, a blank calculation (without oil) was made (KC & Rai, 2007).

Moisture content

Moisture content of the product was determined by the weight loss determination by heating in a thermostatically controlled oven at 105°C for 1 hr (KC & Rai, 2007).

Up to two months of data were analyzed monthly.

The shelf-life tests were carried out once a month based on previous works done on shelf- life of biscuits by

(Rajiv et al., 2012) and (Poudel, 2011; Sangroula, 2018) at Central Campus of Technology.

3. Results

The amount of phytic acid in germinated flaxseed was found to have dropped from an initial content of 10.78±0.03 g/kg to 9.18±0.08 g/kg, respectively. Tannin decreased statistically from a content of 3.34 g/kg to 3.27±0.23 g/kg, respectively (Figure 1)

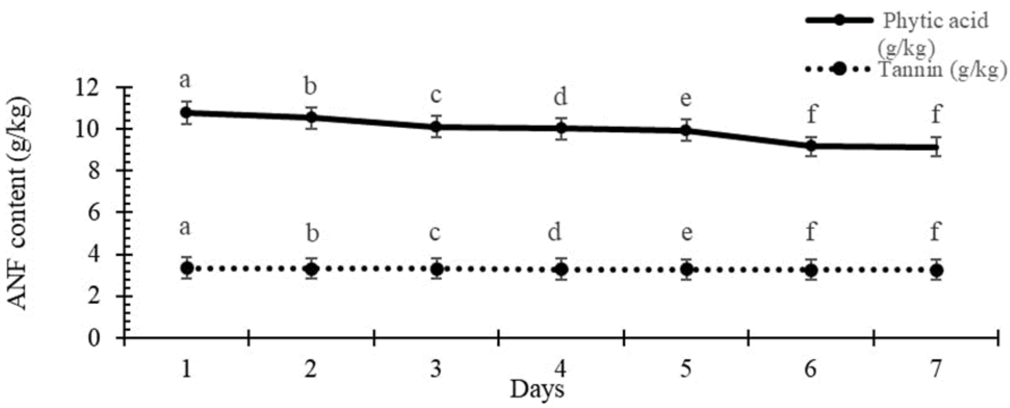
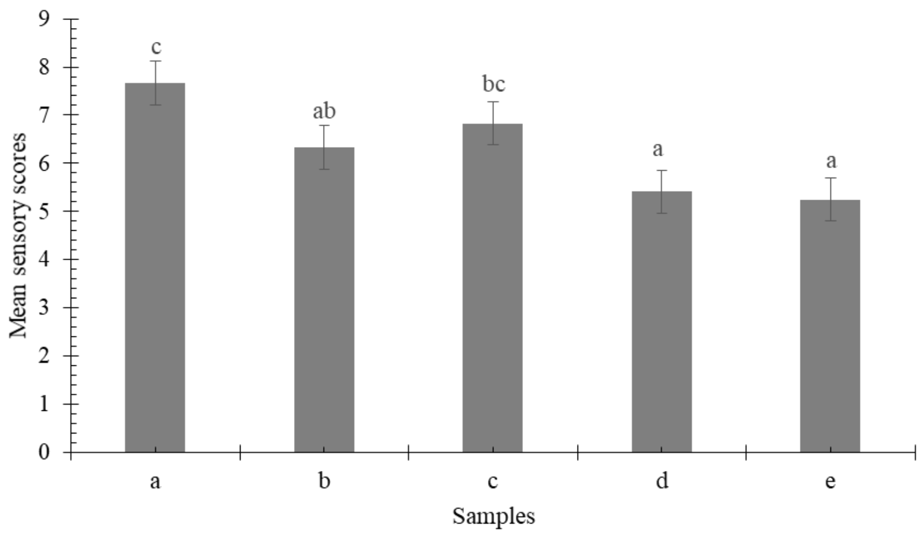


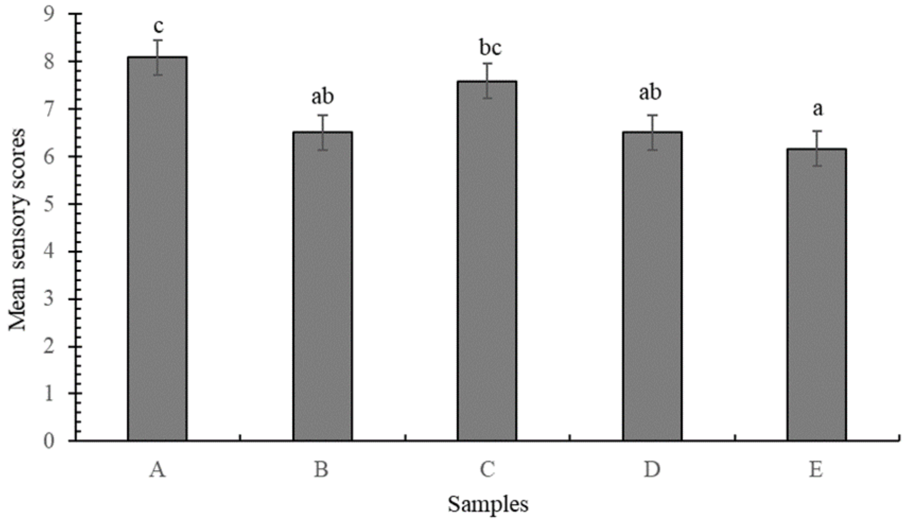
Fig. 1. A line diagram showing the content of anti- nutritional factors (phytic acid and tannins) during germination of flaxseed for 7 days

Based on the mean sensory scores, the formulation with 25 parts germinated flaxseed flour incorporation was chosen for additional physical parameters and physico- chemical analysis. A statistical study revealed that the optimized product's color, crispness, flavor, texture, and general acceptability were significantly impacted by the partial replacement of wheat flour with flaxseed flour ($p < 0.05$). Optimized formulation showed better color, crisper texture, more flavor, better texture, and higher acceptability when compared to other formulations as determined by sensory evaluations.

The study showed that an increased substitution percentage of flaxseed flour resulted in a small increase in biscuit diameter and thickness. The spread ratio of biscuits decreased. As flaxseed flour content increased, the spread ratio for biscuits of different formulations decreased significantly (Figure 2)



(A)



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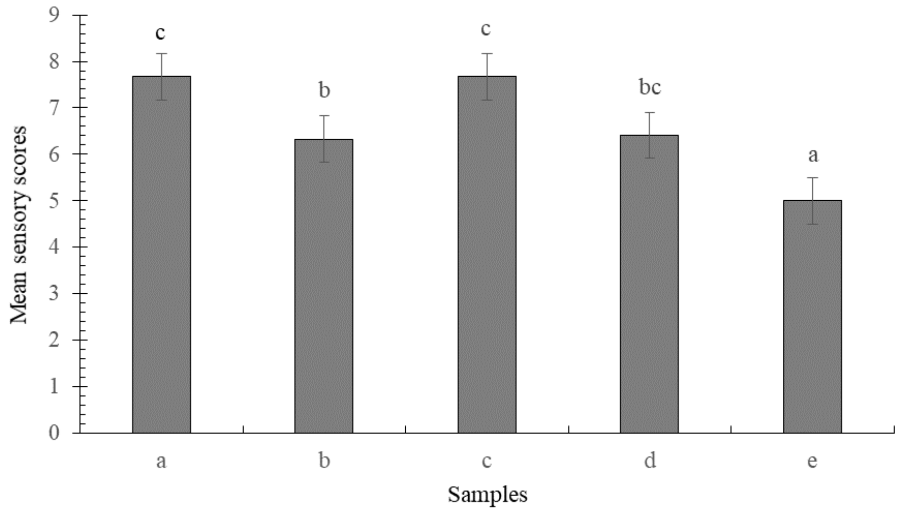
(B)



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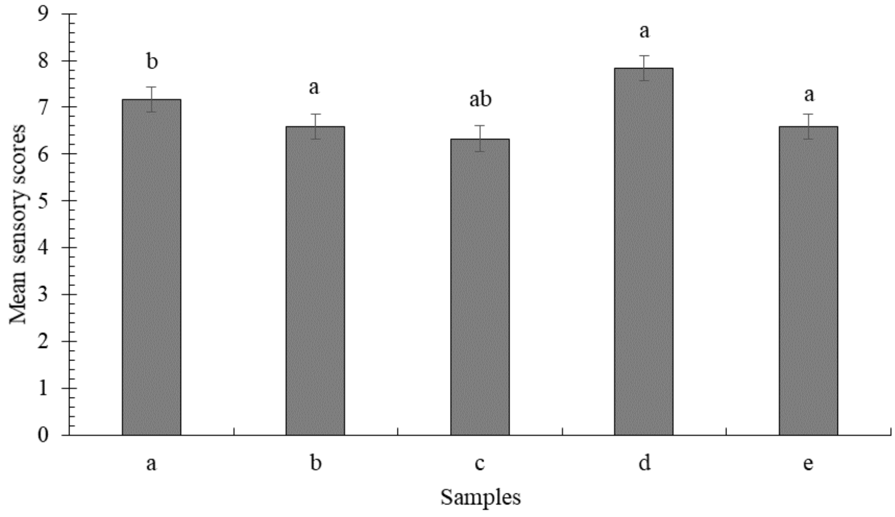
(C)



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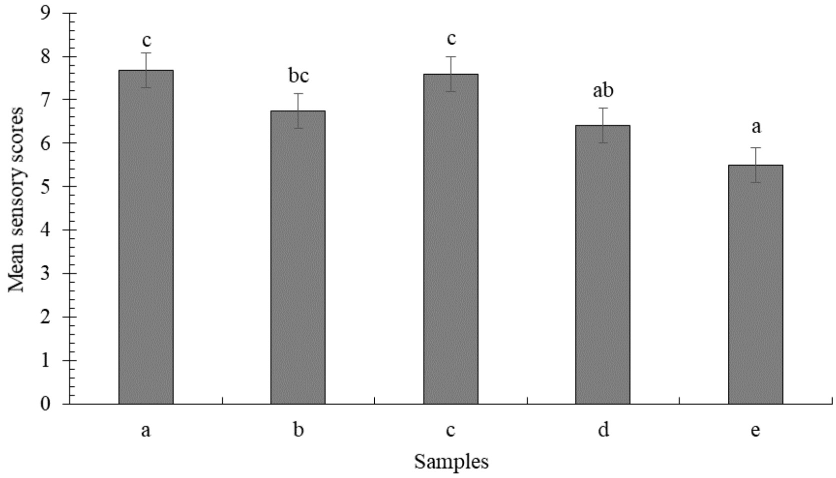
(D)



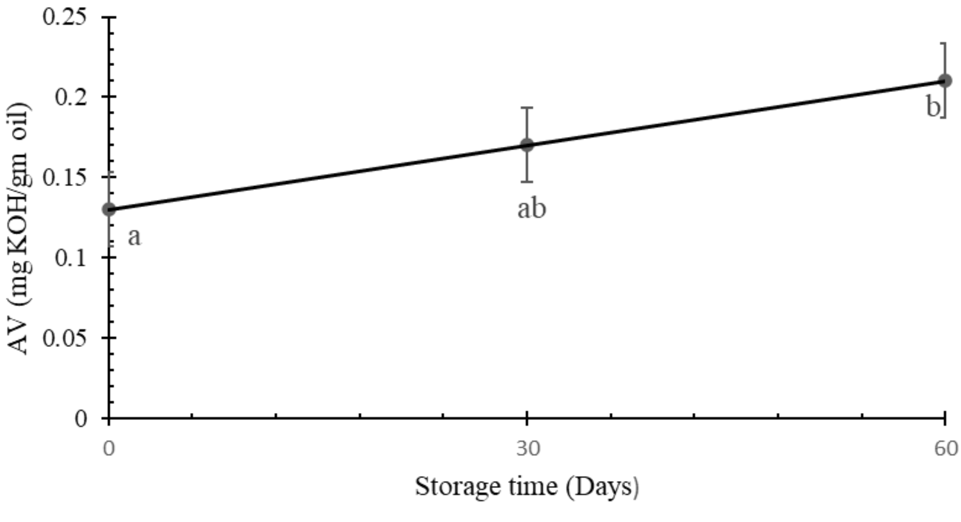
(E)

Fig. 2. Mean sensory scores for colour (A), crispness (B), flavor (C), texture (D) and overall acceptability (E) of flaxseed biscuits

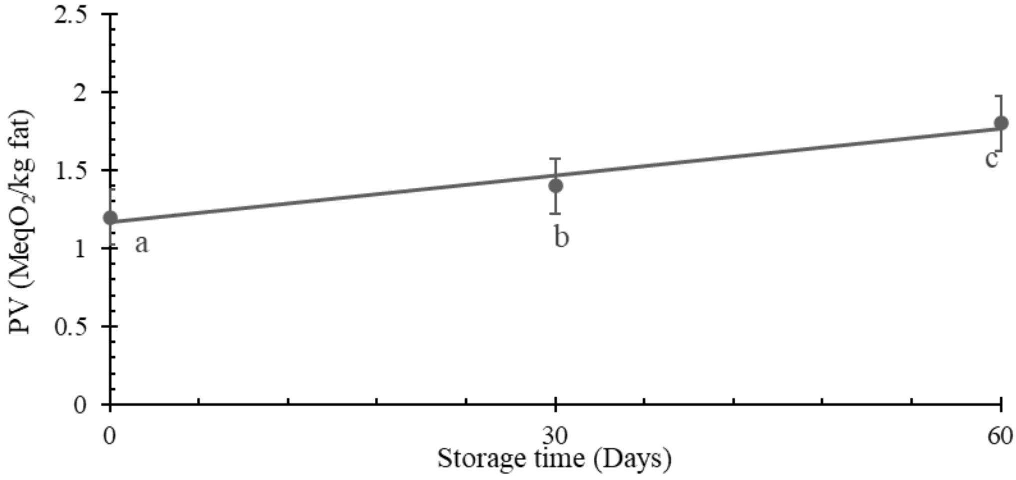
A wheat biscuit contained 2.6% moisture, 1.8% ash, 16.3% fat, 0.7% fiber, 7.8% protein, 73.3% carbohydrates, 1.9 mg/100 gm iron, 20.6 gm/100 gm calcium and 472 kcal in their proximate, ultimate components and energy value, respectively whereas a flaxseed biscuit contained 3.1% moisture, 2.5% ash, 18.3% fat, 3.8% fiber, 13.1% protein, 62.4% carbohydrates, 4.2 mg/100 gm iron, 168.8 mg/100 gm calcium, and 466 kcal as the proximate, ultimate components and energy value.

The moisture content of the flaxseed incorporated biscuit was not significantly higher than that of the wheat flour biscuit ($p > 0.05$). As opposed to this, the ash, fat, fiber, protein, carbohydrates, iron, calcium content, and energy value of the flaxseed biscuits were statistically higher than that of the wheat flour biscuits ($p < 0.05$).

Based on the acid value, peroxide value, and moisture content, the shelf life of the optimized product was investigated (Figure 3). The results were 0.21 mg KOH/g oil, 1.8 MeqO2/kg fat, and 4.88%, respectively, at the end of two months from initial values of 0.13 mg KOH/g oil, 1.2 MeqO2/kg fat, and 3.14% respectively.



(A)



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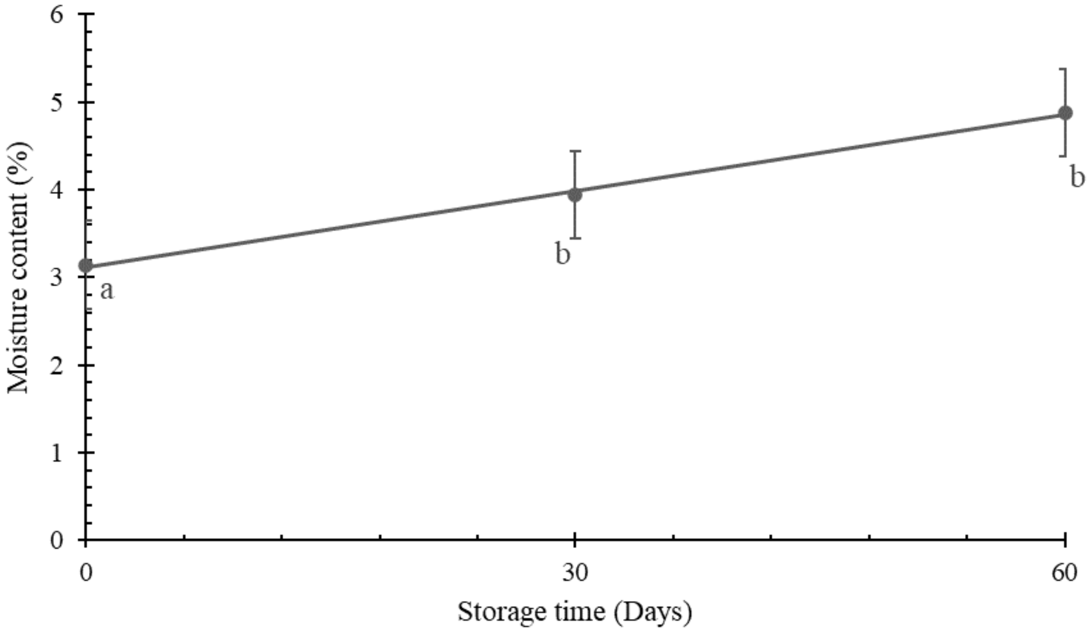

(C)

Figure 3. Changes in acid value (A), peroxide value (B) and moisture content (C) during storage of the flaxseed incorporated biscuits. The significant increase in these values were indicated by a different small letter below the trend lines.

4. Discussion

According to Kajla et al. (2017), the amount of phytic acid in flaxseed decreased from 25.8 g/kg to 21.5 g/kg during germination, which is in agreement with the findings of this study. A slight decline in the tannin concentration of germinated flaxseed was observed as the days since germination increased, confirming the findings of Khandelwal et al. (2010). Khandelwal et al. (2010) also reported that compared to Bengal gram, red gram, and lentil, germinated green gram had a much lower level of total phenolics and tannin.

According to this study, the diameter and thickness of cookies increased as flour replacement increased, supporting the findings of Hussain et al. (2006). In line with the findings of Ganorkar and Jain (2014), who found that dietary fiber and protein have a stronger capacity to bind water, the decrease in spread ratio may be explained by the rise in dietary fiber and protein percentages with increasing levels of flaxseed flour.

When comparing flaxseed and wheat biscuits, it was determined that flaxseed biscuit had higher levels of the proximate (apart from the carbohydrate content) and ultimate components. The findings support the work of Mervat et al. (2015) who reported that except for the total carbohydrates, the chemical composition of cookies significantly changed when flaxseed was added.

A lower iron concentration was measured in the optimized product than that reported by Gupta et al. (2017) of 10.01 mg/100 gm and also, a lower calcium concentration was measured in the optimized product than that reported by Verma et al. (2017) of 170 mg/100gm, while it was higher than that reported by Mekebo and Chandravanshi (2014) as (54-74) mg/100gm. The reason for the lower calcium values could be attributed to flaxseed species and the germination conditions of flaxseed.

In line with the findings of Rajiv et al. (2012), who also reported an increase in acid value, peroxide value, and moisture content of flaxseed-incorporated cookies during (0-90) days of storage, the study revealed an increasing trend in the acid value, peroxide value, and moisture content as storage days progressed.

5. Conclusions

The goal of the current study was to make biscuit incorporated with germinated flaxseed flour and assess its shelf life. According to the study, a biscuit formulation comprising 25 parts (w/w) - germinated flaxseed flour was found to be the most suitable formulation based on the sensory evaluation.

A 7-day germination of flaxseed was shown to have much-reduced levels of tannin and phytic acid. The acid value, peroxide value, and moisture content of the biscuits were found to be within safe levels after two-month shelf-life testing of biscuits. Including 20% of the overhead cost for both biscuits, flaxseed biscuits were priced at Rs. 58 per 100 g, which was more expensive than wheat flour biscuits priced at Rs 53 per 100 g. Overall, flaxseed can be germinated to lessen the anti-nutritional elements and added to a wheat flour biscuit to provide a nutritious and tasty treat.Author's Contribution

Final approval of manuscript was done by all the authors.

Competing interests

The authors declare that they do not have any conflict of interest.

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Ethical Approval and Consent

Verbal consent was obtained from all the respondents for the interview and further publication of the report.

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