CARPATHIAN JOURNAL OF FOOD SCIENCE AND TECHNOLOGY

journal homepage: **http://chimie-biologie.ubm.ro/carpathian_journal/index.html**

Research Article

ULTRASOUND-ASSISTED EXTRACTION OF FLAXSEED MUCILAGE AND ITS APPLICATION AS A FAT AND EGG REPLACER IN CUPCAKES

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https://doi.org/10.34302/crpjfst/2025.17.4.1

Article history:
Received:
May 29th, 2025
Accepted:
October 1st , 2025
Published
December 30th, 2025

Keywords
Ultrasound;
Hot water;
Flaxseed mucilage,
Cupcakes,
Sensory evaluation

Abstract
The present study was designed to investigate the effects of two extraction techniques—conventional hot water extraction and ultrasound-assisted extraction—were compared for flaxseed mucilage (FM) in terms of yield, composition, and antioxidant activity. The yield by hot water extraction technique was 5.30%, while Ultrasound-assisted extraction (from 10 to 30 min) enhanced the yield from 5.25 to 6.95%. Additionally, carbohydrates content decreased, while protein, phenols and lignan contents increased with longer ultrasound treatment. The extracted FM with ultrasound technique showed higher scavenging ability on DPPH and ABTS radical. This research supports the development of functional, value-added flaxseed products and highlights the potential used of FM as a novel food hydrocolloid, including and its application as fat and egg replacer in cupcakes.
Cupcakes were prepared with varying substitution levels (25, 50, 75, and 100%) from egg and oil, and their sensory, physical, and textural properties were evaluated. Results showed that 25–50% replacement levels provided acceptable quality and consumer acceptability, demonstrating the functional viability of flaxseed mucilage in cupcakes. In general, the replacement of FM (25%) as fat or egg replacement in cupcake formulation showed higher scores in all sensory attributes as compared to the control. Results demonstrated that flaxseed mucilage enhanced the total phenolic content of cupcakes when used as a substitute for fat, eggs, or both.

1. Introduction

Flaxseed is classified as one of the most important functional foods because of its content of polyunsaturated acid (Omega 3), lignan, dietary fiber, phenols compounds, protein and mucilage (Azarpazhooh et al., 2021). Mucilage consists of biologically interesting macromolecules rich in polysaccharides. It dissolves when it comes into contact with water and produces a viscous substance, like gelatin. Mucilage has good functional properties and used as food coatings, edible films, smart packaging, pharmaceutical gels, encapsulation material and fat replacer (Prajapati et al., 2013; Elsorady, 2016; Mohtarami et al., 2022; Elsorady et al., 2024a,2024b). Mucilage has an important role in food industry because of its emulsifying properties. Flaxseed mucilage (FM) is a valuable hydrocolloid with functional properties similar to those of gum arabic. FM exhibits excellent rheological properties and strong oil- and water-binding capacity. It contains 50–80% carbohydrate, 4–20% protein, and 3–9% ash (Puligundla and Lim, 2022).

FM is widely used in food as a texturing agent due to its thickening properties. It is used as a lubricant for skin surface in cosmetic and pharmaceutical applications. Food contains FM can prevent intestinal inflammation and decreasing blood glucose and blood cholesterol (Fabre et al., 2015). FM is one of the by-products of flaxseed about 3–10%. Its composition depends on variety, cultivation practices, and environmental conditions. FM possesses valuable functional properties—such as water-holding capacity, emulsifying ability, fat replacement, texture modification, stabilization, and interfacial activity—which make it an essential ingredient in diverse food applications, including gluten-free baked goods, plant-based meat and dairy alternatives, salad dressings, edible gels, and emulsions (Chand et al., 2024).The yield, purity, and functional properties of FM depend on extraction method and temperature (Lorenc et al., 2022). The most common method for FM extraction is aqueous extraction which is not considered time-and energy efficient. So, the best emerging technique is sonication due to its simplicity, cost efficiency, and optimum extraction yield with fixed biological properties. Sonication has various advantages such as low temperature and energy requirements, lower extraction time, and improve quality of extracted material. Ultrasonic waves disrupt tissues via physical forces developed during the cavitation process, which accelerate the extraction of organic compounds, contained within the body of plants and enhanced mass transfer of cell contents in a short time (Hromádková et al., 1999, Chand et al., 2024). Several studies have demonstrated the benefits of ultrasonication in extracting bioactive compounds, such as antioxidants and polysaccharide gums, from plant-based sources (Akhtar et al., 2019; Chemat et al., 2020; Kumar et al., 2021; Manzoor et al., 2021).

Cakes are among the most widely consumed bakery products worldwide, appreciated by people from diverse backgrounds. Cakes structure is often made up of gelatinized starch particles enclosed in foam-forming egg white protein. The proteins that give cakes their strength and shape were present in both flour and eggs. The egg's and cake flour's structural strengthening effects are counterbalanced by the sugar and fat ingredients' tenderizing effects (Salem et al., 2024)

Hydrocolloids are proteins or polysaccharides which have functional properties such as thickening, gelling, stabilizing, film forming, dispersing and texture modifying that are successfully used in food products (Elsorady, 2016). FM is used in the food industry to enhance stability, increase viscosity, improve emulsifying ability, and provide desirable rheological and foaming properties (Mueed et al., 2022). Consumers have become more health conscious in recent years. For that, there is a growing trend for rising low-fat foods as consumers are increasing looking to modify their dietary intake to reduce fat (Yang et al., 2007). Fat replacers are commonly used in baked products, offering similar functional properties to fat but with fewer calories (Mohtarami et al., 2022). Egg is one of the most used and consumed ingredients in food applications (Miranda et al., 2015). It is usually used into food to improve sensory, emulsifying, foaming and gelling properties (Abeyrathne et al., 2013; Gallo et al., 2020). However, with the increasing demand for vegan options and food allergies to eggs (Gallo et al., 2020), FM was explored as fat and egg replacer in cupcakes. This study aimed to extract FM using ultrasound-assisted techniques and to evaluate its effectiveness as a fat and egg replacer in product cupcake by fortified with flaxseed mucilage extraction to become a functional healthy food high in bioactive components

2. Materials and methods

2.1. Materials

Flaxseed meal (Giza12), was obtained as byproduct after oil extraction, was defatted. All chemicals and reagents were obtained from Sigma-Aldrich (St.Louis, USA) and El-Gomhoria Co. for Pharmaceutical (Alexandria, Egypt).

2.2. Methods

2.2.1. Flaxseed mucilage (FM) extraction

FM was extracted by hot water extraction and ultrasound-assisted extraction.

2.2.1.1. Hot water extraction

FM was extracted from flaxseed meal using hot water extraction according to Elsorady et al. (2024a) with minor modification. Flaxseed meal was soaked in distilled water (1:8 w/v) with continuous stirring (2 hr) at 90 °C. Then soaked meal was filtered, centrifuged (4,000 rpm, 10 min). The precipitated gum was lyophilized.

2.2.1.2. Ultrasound treatment

Ultrasound-assisted extraction was conducted according to Mahmod et al. (2023), with minor modifications. Flaxseed meal was sonicated by ultrasonic waves, a SELECTA ultrasonic bath. Flaxseed meal was bottled with distilled water (1:8 w/v) and put into bath filled with distilled water. Flaxseed meal was treated with ultrasound waves for various times (10, 15, 20, 25 and 30 minutes). The soaked mixture was then filtered and centrifuged (4,000 rpm, 10 min). The precipitated mucilage was subsequently lyophilized.

2.2.2. Effect of extraction method on yield and composition of FM

The yields and compositions of the FM samples extracted with different methods were compared.

2.2.2.1 Yield of flaxseed mucilage

Extracted mucilage yield was calculated as follows:

$$\text{Yield } (\%) = (G / S) \times 100 \quad (1)$$

where 'G' is the weight of dried mucilage after extraction from Flaxseed meal and 'S' is Flaxseed meal weight (Elsorady et al., 2024a).

2.2.2.2. Proximate composition of FM

Moisture, protein, fiber, and ash contents were determined according to AOAC (2010). Total carbohydrates were determined by difference. Caloric value (Kcal/ 100g) was calculated from the sum of the percentages of crude protein and total carbohydrates multiplied by a factor of 4 plus the crude fat content multiplied by 9 according to Zambrano et al., (2004).

2.2.2.3. Total phenols content

Using Folin–Ciocalteu reagent, total phenol content was colorimetrically determined at 725 nm as described by Elsorady et al. (2024a).

2.2.2.4. Lignan extraction

Lignan content (%) was determined using the method described by Elsorady et al. (2024a).

2.2.3. Effect of extraction methods on antioxidant activity of FM

2.2.3.1 DPPH scavenging activity

The scavenging ability on 2, 2-diphenyl-1-picryl hydrazyl (DPPH) radical was determined according to Elsorady and Abdl Aziz (2011) with minor modifications. The extracted FM by different methods (5, 10, 15, 20, 25, and 30 mg/ml) was dissolved in distilled water (0.5 ml) and mixed with 1 ml of methanolic solution including DPPH radicals, resulting 0.2 mM DPPH as end concentration. The mixture was agitated, and kept in dark at 22 °C, and the absorbance was then estimated at 517 nm against a blank. The scavenging ability was calculated as follows:

$$\text{Scavenging ability } (\%) = [(\Delta\ A517 \text{ of control} - \Delta\ A517 \text{ of sample}) / \Delta\ A517 \text{ of control}] \quad (2)$$

2.2.3.2. ABTS scavenging activity

The scavenging ability on ABTS was determined according to Liang et al. (2017). ABTS (7 mM) and potassium persulfate ($K_2S_2O_8$, 2.45 mM) solutions were mixed and incubated in the dark for 24 hours, then diluted with 10 mM phosphate buffer (pH 7.4) until the absorbance at 734 nm reached approximately 0.700.of FM {30 µL of various amounts (5, 10, 15, 20, 25, and 30 mg)} were blended with diluted solution of ABTS and absorbance at 734 nm was measured before and after adding FM. The scavenging ability was measured as follows:

Scavenging ability (%) = [(Δ A734 of control – Δ A734 of sample) / Δ A734 of control].

(3)

2.2.4. Preparation of Cupcakes blends

Cupcakes were prepared following the formulations in Table 1, using a procedure adapted from Moiraghi et al. (2013) with slight modifications.. Egg and oil were substituted with FM solution after filtration at ratios 25, 50, 75 and 100 %, all ingredients have been previously equilibrated at room temperature. Then, oil and FM were creamed with sugar using Moulinette machine (Model 320, cod 25, France) for 8 minutes. Next, the rest of the wet ingredients were added followed by dry ingredients and mixed under constant mixing. The dough was transferred to greased baking pans and baked in an oven at 160°C for 35 minutes. After baking, the cupcakes were removed from the pans, left for 1 h at 25°C to cool and then packed in plastic bags to prevent drying. Cupcake evaluation was conducted after 24 hours of storage at room temperature. All treatments were prepared in laboratories Home Economics Department, Faculty of Specific education, Kafrelsheikh Univ., Kafrelsheikh, Egypt.

Table 1. Formulation of Cupcakes with different levels of FM as fat and egg replacers.

Ingredients	**FM as fat replacer**				
	Control	25%	50%	75%	100%
Flour (g)	250	250	250	250	250
Sugar (g)	100	100	100	100	100
Oil (ml)	100	75	50	25	-
FM (ml)	-	25	50	75	100
Egg (g)	150	150	150	150	150
Vanilla (g)	1	1	1	1	1
Baking powder (g)	3	3	3	3	3
Milk (mL)	125	125	125	125	125
Ingredients	**FM as egg replacer**				
	Control	25%	50%	75%	100%
Flour (g)	250	250	250	250	250
Sugar (g)	100	100	100	100	100
Oil (ml)	100	100	100	100	100
Egg (g)	150	112.5	75	37.5	-
FM (ml)	-	37.5	75	112.5	150
Vanilla (g)	1	1	1	1	1

Baking powder (g)	3	3	3	3	3
Milk (mL)	125	125	125	125	125

Ingredients		**FM (No egg and No fat)**
Flour (g)	250	250
Sugar (g)	100	100
Oil (ml)	100	-
Egg (g)	150	-
FM (ml)	-	250
Vanilla (g)	1	1
Baking powder (g)	3	3
Milk (mL)	125	125

2.2.5. Sensory evaluation

Sensory evaluation was conducted on control cupcakes and those containing flaxseed mucilage as fat and egg replacers, using a panel composed of staff and students (both male and female) from the Home Economics Department, Kafrelsheikh University. The panelists evaluated the cupcakes for color, uniformity, softness, odor, taste and overall acceptability. Each sensory attribute was rated on a 5 point hedonic scale (1 = very poor, 2 = poor, 3 = moderate, 4 = good and 5 = very good) (Noshad et al., 2022).

2.2.6. Physical properties and Water Activity

Weight (g), height (cm), volume (cm^3) was measured at room temperature (AACC, 1983). The specific volume was determined as the ratio of volume to weight (Pong et al., 1991). Water activity of cupcakes was determined by a Rotronic Hygro Lab EA10-SCS apparatus (Switzerland). Each sample was analyzed in triplicate.

2.2.7. Proximate composition, caloric value and total phenols of cupcake

Moisture, protein, fibers, and ash were determined according to AOAC, (2010). Total carbohydrates were determined by difference. Caloric value was calculated as the following equation according to Zambrano et al., (2004):

$$\text{Caloric value (Kcal/ 100g)} = 4(P+C) + 9F \quad (3)$$

Where P for proteins, C for carbohydrates and F for fats.

The methanolic extract of cupcakes was prepared as described by Giri et al. (2024) [1 g sample was prepared in 10 ml of methanol and kept for overnight, followed by shaking for 3 h, centrifugation (for 15 min, 10,000 rpm)]. Total phenols content (TPC) of the methanolic extract was determined colorimetrically using Folin-Ciocalteu method (Elsorady 2024a).

2.2.8. Color properties

The colors of cupcakes samples were determined by the colorimeter (Minolta model CR-410, Tokyo, Japan). Results were expressed as lightness-darkness (L), greenness-redness (a), and blueness-yellowness (b) (Mohtarami, 2019).

2.3. Data analysis

Data were illustrated as mean and statistical analysis was performed using ANOVA test by SPSS (V16). The values P < 0.05 were considered as statistically significant.

3. Results and discussions

3.1. Effect of extraction method on yield and proximate composition of FM

The extraction yield of FM by hot water extraction (conventional method) was 5.30% (Table 2). According to Safdar et al. (2020), higher temperatures enhance solute solubility and diffusion rates, leading to greater mass transfer and improved extraction efficiency.

The effect of ultrasound extraction time on FM yield is presented in Table 2. Results revealed that increasing ultrasound time significantly affected the yield of FM. Extending ultrasound treatment from 10 to 30 minutes increased the FM yield from 5.25% to 6.95%. The mechanical disruption of cell walls resulting in increased accessibility (Ying et al., 2011).These results agreed with that obtained by Safdar et al. (2020).

Table 2. Effect of extraction method on yield and proximate composition of FM*

Parameters	Hot water extraction	Ultrasonic assisted extraction				
		10 min	15 min	20 min	25 min	30 min
Yield (%)	5.30^{d}	5.25^{d}	5.83^{c}	6.11^{bc}	6.32^{b}	6.95^{a}
Moisture	5.11^{c}	5.56^{ab}	5.51^{b}	5.54^{b}	5.16^{c}	5.76^{a}
Protein	9.52^{d}	8.29^{e}	9.35^{d}	10.84^{c}	11.76^{b}	13.63^{a}
Ash	4.84^{d}	4.78^{d}	5.43^{c}	5.92^{b}	6.23^{ab}	6.47^{a}
Carbohydrates	80.53^{b}	81.37^{a}	79.71^{b}	77.70^{c}	76.25^{c}	74.25^{d}

*different superscripts indicate significant differences using Duncan's multiple range tests (p < 0.05)

During ultrasound treatment, the ultrasound waves create acoustic cavitation which leads to cell wall rupture and creates pores through them. These lead to high penetration of solvents into the cellular matrix which, in turn, facilitate and accelerate mass transfer (Vinatoru, 2001).

Effect of ultrasonic assisted extraction method on proximate composition of FM was illustrated at Table (2). Carbohydrates were decreased from 81.37 to 74.25% with increment time of extraction up to 30 min. Also, ultrasonic assisted extraction for 10 min increased the carbohydrates content as compared to that extracted with hot water extraction. Also, Safdar et al. (2020) observed that carbohydrates were decreased with with increasing ultrasonic extraction time up to 30 min. Wang et al. (2022) indicated that the carbohydrate content of chia mucilage decreased significantly with extraction time and temperature. It may be related to several compounds (for example, insoluble substances) that could be suspended in the extraction mixture when the cells of the raw material ruptured and caused subsequent low permeation of the solvent (Maran et al., 2013), resulting in higher yield but lower FSG purity with the increasing extraction time. Furthermore, the protein and ash content of mucilage was higher when the extraction time and temperature rose, the proportion of carbohydrates was relatively low. Furthermore, protein was increased from 8.29 to 13.63% with increasing time from 10 min to 30 min. It is related to small molecules of protein than carbohydrates and more intense in deeper layers of flaxseed. Ultrasounds do not promote the release of the protein content but by increasing ultrasonic treatment time, the seeds begin to become de-structured, and proteins may therefore be more easily extracted (Fabre et al., 2015; Ying et al., 2011). The results were agreed with those obtained by Fabre et al. (2015), Wang et al., (2022). Farahnaky et al. (2013) mentioned that, the microscopic shearing forces generated by ultrasonic wave can damage seed coat, and more protein and ash is released to the aqueous solution as the extraction time increased.

Data also indicated that there is no significant difference in carbohydrates and protein contents between FM extracted by hot water extraction and that extracted by ultrasonic assisted extraction for 15 min. Furthermore, there is significant difference in yield of FM between that extracted by hot water extraction and that extracted by ultrasonic assisted extraction for 15 min.

3.2. Effect of extraction method on bioactive components of FM

Also data indicated that total phenols and lignan contents in FM were increased with increasing time extraction (Table 3). Total phenols and lignan contents of FM prepared with ultrasound extraction for 30 min was significantly higher than that of FM prepared with hot water extraction.

Table 3. Effect of extraction method on bioactive components of FM*

Bioactive components	Hot water extraction	Ultrasonic assisted extraction				
		10 min	15 min	20 min	25 min	30 min
Total phenols (mg GAE/100 g)	19.25^{d}	15.24^{f}	17.62^{e}	19.67^{c}	20.41^{b}	22.30^{a}
Lignans (%)	3.54^{d}	2.35^{f}	2.89^{e}	3.64^{c}	3.93^{b}	4.51^{a}

*different superscripts indicate significant differences using Duncan's multiple range tests ($p < 0.05$)

Linseed mucilage (LM) shows good antioxidant quantity as it is rich in phenolic compounds such as ellagic acid, cinnamic acid, caffeic acid, epicatechin, and vanillic acid. This behavior of LM positively impacts the gut as it helps synthesize α-glucosidase inhibitors and lipase, which in turn regulate the metabolism of the GIT tract (Chand et al., 2024). Other supporting studies associated with mucilage antioxidant activity were recorded in okra, quince seed mucilage, and Opuntia ficus-indica [Jouki et al., (2013); Nampuak and Tongkhao, (2020)]. Also, many studied indicated that ultrasound assisted extraction increased the extracted yield of phenols components from plants [Ma et al., (2008); Wang et al., (2008); Taha et al., (2011)]

3.3. Effect of extraction method on Antioxidant activity of FM

Antioxidant activity of FM was determined by DPPH and ABTS tests. The antioxidant of FM can react with free radicals DPPH and change the purple color of this substance to yellow by reducing the stability of free radicals. The ability of DPPH to scavenge free radicals is measured by the amount of color change produced. The results showed that the DPPH scavenging activity of FM extracted by Ultrasonic assisted extraction was higher than those extracted by hot water extraction (Table 4). As shown, DPPH scavenging activity increased with FM concentration increment. It is may be related to bioactive components in FM. Also, it may be related to the ability of polysaccharide to release hydrogen/electrons that elevated with increasing concentration to terminate the chain reaction of the free radicals that could be the possible reason (Wang et al., 2017). These results agreed with Safdar et al. (2020) and Akhtar et al. (2023).

Table 4. Effect of extraction method on antioxidant activity of FM*

Antioxidant activity	FM (mg/ml)											
	Hot water extraction						Ultrasonic assisted extraction for 30 min					
	5	10	15	20	25	30	5	10	15	20	25	30
DPPH scavenging (%)	**45.22^{l}**	**72.32^{j}**	**75.59^{i}**	**85.42^{f}**	**90.40^{d}**	**92.52^{c}**	**53.31^{k}**	**76.42^{h}**	**78.51^{g}**	**88.31^{e}**	**93.45^{b}**	**96.35^{a}**
ABTS scavenging (%)	**22.52^{l}**	**28.34^{j}**	**32.57^{i}**	**48.36^{f}**	**58.63^{d}**	**63.54^{b}**	**23.21^{k}**	**35.41^{h}**	**40.18^{g}**	**51.33^{e}**	**62.60^{c}**	**68.68^{a}**

*different superscripts indicate significant differences using Duncan's multiple range tests ($p < 0.05$)

The ABTS radical scavenging activity is a decolorizing technique that is applicable for water and lipid soluble antioxidant capacity, generally used in natural components (i.e. polysaccharides and polyphenols) (Wang et al., 2017). The scavenging ability was higher in FM extracted by Ultrasonic assisted extraction than that extracted by hot water extraction (Table 4).Date showed that the ABTS scavenging ability was increased by increasing the concentration of FM concentration. These results agreed with Safdar et al. (2020) and Akhtar et al. (2023).

FM has antioxidant activity contra ABTS and DPPH. Whereas the antioxidant activity of FM contra DPPH are primarily effected by the content of hydroxycinnamic acid (mostly p-Coumaric acid), other phenols components are effective contra ABTS, closely related to the content of phenols (Kučka et al., 2024). These results develop the functional and value-added products from flaxseed.

Figure 1. Cupcakes processed with FM at different ratios

3.4. Effect of replacement ratios of FM on sensory evaluation of cupcakes

Incorporating flaxseed mucilage as a partial substitute for eggs or fat in cupcakes resulted in noticeable changes in the product's quality attributes (Figure 1). At 25% and 50% replacement levels, the cupcakes maintained a soft crumb, satisfactory volume, and desirable moisture content, closely resembling the control sample. These samples also received favorable scores in sensory evaluation, particularly in taste and texture. However, higher substitution levels (75% and 100%) led to a denser texture and slight flavor deviations, affecting consumer preference. These outcomes suggest that mucilage substitution at moderate levels preserves cupcake quality while enhancing its nutritional profile.

Sensory attributes have important role in assessing product acceptability (Hesarinejad et al., 2019). The sensory evaluation of cupcakes using FM as fat and egg replacers was evaluated in terms of color, uniformity, softness, odor, taste and overall acceptability (Table 5). Results indicated that cupcakes produced containing FM as fat replacers at 25% and as egg replacers at 25% showed higher scores in all sensory attributes as compared to the control.

Table 5. Effect of replacement ratios of FM on sensory evaluation of cupcakes*

Attributes	**FM as fat replacer**				
	Control	25%	50%	75%	100%
Color	4.25$^{a b}$	4.50^{b}	3.75$^{a b}$	3.75$^{a b}$	3.50^{a}
Uniformity	4.00$^{a b}$	4.25^{b}	3.75$^{a b}$	3.50$^{a b}$	3.25^{a}
Softness	4.00^{a}	4.75^{b}	4.75^{b}	4.75^{b}	4.75^{b}
Odor	4.25^{b}	4.50^{b}	3.75$^{a b}$	3.25^{a}	3.25^{a}
Taste	4.50^{c}	4.75^{c}	3.75^{b}	3.25$^{a b}$	3.00^{a}
Overall acceptability	4.50$^{b c}$	4.75^{c}	3.75$^{a b}$	3.75$^{a b}$	3.5^{a}
Attributes	**FM as egg replacer**				
	Control	25%	50%	75%	100%
Color	4.25$^{a b}$	4.75^{b}	4.00^{a}	4.00^{a}	3.75^{a}
Uniformity	4.00$^{a b}$	4.50^{b}	4.25$^{a b}$	3.75$^{a b}$	3.50^{a}
Softness	4.00^{a}	4.75^{b}	4.75^{b}	4.75^{b}	4.75^{b}
Odor	4.25$^{a b}$	4.75^{b}	4.00$^{a b}$	3.50^{a}	3.50^{a}
Taste	4.50^{b}	4.75^{b}	4.00$^{a b}$	3.50^{a}	3.50^{a}
Overall acceptability	4.50$^{b c}$	4.75^{c}	4.00$^{a b c}$	3.75$^{a b}$	3.50^{a}
Attributes	**FM (No egg and No fat)**				
Color	4.25^{a}	3.50^{a}			
Uniformity	4.00^{b}	3.25^{a}			
Softness	4.00^{a}	4.75^{b}			
Odor	4.25^{a}	3.50^{a}			

Taste	4.50 b	3.00 a
Overall acceptability	4.50 b	3.00 a

*different superscripts indicate significant differences using Duncan's multiple range tests ($p < 0.05$)

The sensory evaluation of cupcakes produced containing FM as fat and egg replacers (No egg- No fat) had lower scores in all sensory attributes as compared to control sample.

After sensory appreciation of the control sample, Cupcakes using FM as Fat replacer (25%) , Cupcakes using FM as egg replacer (25%) and Cupcakes using FM as fat and egg replacers (No egg- No fat) were selected for further analyses to assess some physical properties and chemical composition.

3.5. Effect of replacement ratios egg and oil levels with different levels of FM on some physical properties of Cupcakes

The data regarding height, weight, volume, specific volume, water activity and baking loss weight of cupcakes prepared with FM as fat and egg replacements are illustrated in Table 6. Using FM as egg replacer and both fat and egg replacers enhanced height, weight, volume and specific volume as compared with control sample. Also, results revealed that using FM increased water activity of cupcakes. It may be related to good water holding capacity of FM. Addition of hydrocolloids may increase the air bubble distribution in cupcakes and help to retain that air bubble in the cake during baking (Andrade et al., 2018; Ozkoc and Seyhun, 2015). Hussain et al. (2013) indicated that flaxseed meals improved air retention and enhanced batter aeration.

Table 6. Effect of replacement ratios egg and oil levels with different levels of FM on some physical properties of cupcakes compared to cupcakes (control)*

Cupcakes samples	Height (cm)	Weight (g)	Volume (cm^3)	Specific volume	Water activity
Cupcakes (Control)	3.80^{ab}	50.40^{c}	122.18^{a}	2.42^{a}	0.854^{d}
Cupcakes (75% oil : 25% mucilage as fat replacer)	3.50^{b}	47.33^{d}	112.54^{b}	2.38^{b}	0.875^{c}
Cupcakes (75% egg : 25% mucilage as egg replacer)	3.80^{ab}	53.21^{b}	129.94^{a}	2.44^{a}	0.895^{b}
Cupcakes (Mucilage as no egg and fat replacers)	4.30^{a}	55.80^{a}	138.26^{a}	2.48^{a}	0.905^{a}

*different superscripts indicate significant differences using Duncan's multiple range tests ($p < 0.05$)

3.6. Effect of replacement ratios egg and oil levels with different levels of FM on chemical composition of Cupcakes

FM was replaced to cupcakes ingredients as fat and egg replacements. When FM was used as a fat replacer, the fat content decreased by 17.11%, 5.28% as egg replacement, 92.12% as both egg and fat replacements. Similarly, FM increased the carbohydrate content in cupcakes, which improved their nutritional profiles. Results were in accordance with that obtained ay Ahmad et al., (2021). Felisberto et al. (2015)

indicated that moisture is a desirable quality often associated with the softness of a baked product.

Control sample showed lower moisture, carbohydrates contents and higher oil content and caloric value than other samples (Table 7). As expected, significant increases in moisture, carbohydrates contents were obtained in the cupcakes using FM is related to good water-holding property (Chand et al., 2024) and chemical composition of FM (Table 2). Also, FM efficiency as fat and egg replacers in cupcakes was assessed based on caloric values of all formulated cupcakes. The calorie value ranged from 398.03 to 506.25 kcal. The calorie value of cupcakes was decreased by replacing fat and egg with FM. Using FM as fat or egg replacers in cupcakes plays an important role in reducing calories value. It was agreed with that obtained by Ahmad et al., (2021).

Table 7. Effect of replacement ratios egg and oil levels with different levels of FM on chemical composition of Cupcakes (on dry weight)*

Cupcakes samples	Moisture	Crude protein	Fat	Ash	Total Carbohydrates	Caloric value (Kcal/100g)	Reduction of calories (%)
Cupcakes (Control)	35.56^{c}	8.31^{b}	22.73^{a}	1.85^{c}	67.11^{c}	506.25^{a}	-
Cupcakes (75% oil : 25% mucilage as fat replacer)	36.56^{c}	9.66^{a}	18.84^{b}	2.04^{b}	69.46^{b}	486.04^{b}	3.99
Cupcakes (75% egg : 25% mucilage as egg replacer)	38.10^{b}	7.75^{c}	21.53^{a}	1.70^{c}	69.02^{b}	500.85^{a}	1.08
Cupcakes (Mucilage as no egg and fat replacers)	48.49^{a}	9.50^{a}	1.79^{c}	2.73^{a}	85.98^{a}	398.03^{c}	21.38

*different superscripts indicate significant differences using Duncan's multiple range tests ($p < 0.05$)

Moisture content was increased with replacing fat and egg with FM. This is due to the water holding property of FM (Mueed et al., 2022). Similar results were reported by Elsorady et al., (2024b) when using FM as bread improver or oil replacers in pan bread. The results were also consistent with the study of Fernandes and Salas-Mellado (2017) when chia mucilage was used as a fat substitute in cake and bread.

Figure 2 illustrates the effect of using FM as a fat or egg replacer on the total phenolic content (TPC) of cupcakes. As the level of FM substitution increased—whether for fat, egg, or both the TPC of the cupcake samples increased significantly compared to the control. Specifically, the TPC increased by 1.90, 1.98, and 3.92 times in samples using FM as fat, egg, and combined fat-and-egg replacers, respectively. These increases are attributed to the presence of phenolic compounds naturally found in FM. This finding aligns with previous findings by Mohtarami et al. (2022) and Ahmadinia et al. (2023), reinforcing the potential of FM to enhance the benficial nutritional profile of baked products through its bioactive components.

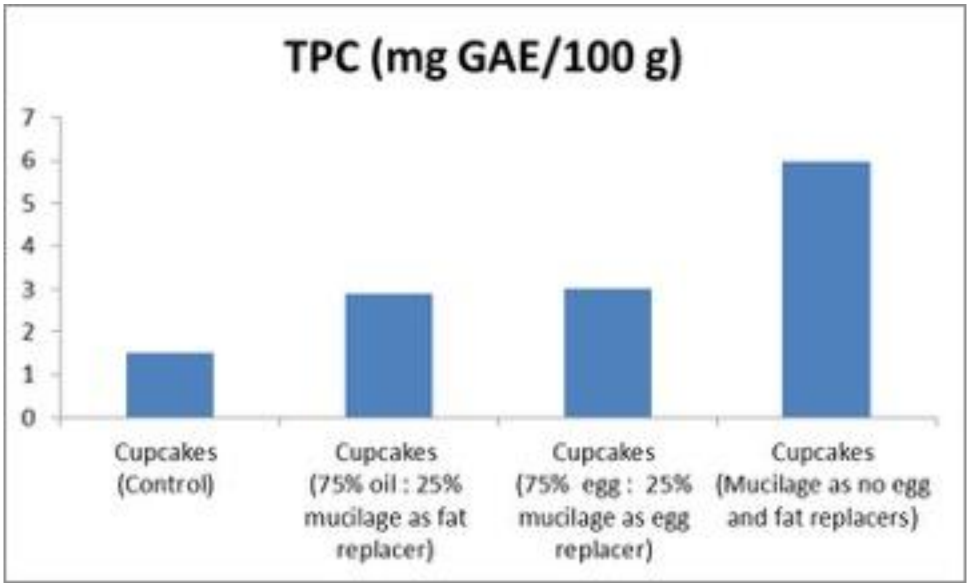

Figure 2. Total phenols content (TPC) in cupcake samples.

3.7. Effect of replacement ratios egg and oil levels with different levels of FM on color of Cupcakes

Color is one of the most important qualitative parameter of bakery product that influences consumer sensory acceptance. It is affected by several factors such as ingredients, baking conditions and reactions that are occurred during baking (Maillard reaction and caramelization) (Ameur et al., 2007). Table (8) presents L, a, and b color values for the crust and crumb of cupcake samples. The results of color analysis of cupcakes using FM as fat and egg replacers showed that using FM significantly increased brightness (L) of cupcakes for crust and crumb, and significantly reduced redness (a) and yellowness (b) for crust and crumb of cupcakes. The increase of L may be related to the lighted color of FM. Salehi (2018) indicated that the increase of balangu seed gum increased the amount of lightness (L*) which may be related to increase in the cake's volume, which makes the internal texture of the cakes brighter. Increasing L* value may be related to retention of moisture by the gums (Lazaridou et al., 2007). Retaining moisture during baking reduces crust changes in bakery products. The lightening of cake was due to changes of crust. Uniform crust more than non-uniform crust enhanced L* value (Lazaridou et al., 2007). Results were in agreement with those obtained by Elsorady et al. (2024b) of using FM as bread improver and fat replacers in pan bread and Mohtarami et al. (2022) as using FM as fat replacer on cookies.

Table 8. Effect of replacement ratios egg and oil levels with different levels of FM on color of Cupcakes*

Cupcakes samples	Crust			Crumb		
	L	a	b	L	a	b
Cupcakes (Control)	55.24[d]	12.03[a]	29.10[a]	68.13[d]	-0.41[a]	17.38[a]
Cupcakes (75% oil : 25% mucilage)	55.83[c]	11.61[b]	28.41[b]	70.72[c]	-0.46[b]	17.33[b]
Cupcakes (75% egg : 25% mucilage)	56.26[b]	11.40[c]	27.71[c]	71.12[b]	-0.50[c]	17.23[c]
Cupcakes (Mucilage as no egg and fat replacers)	57.21[a]	11.22[d]	26.14[d]	72.30[a]	-0.55[d]	17.13[d]

*different superscripts indicate significant differences using Duncan's multiple range tests ($p < 0.05$)

The substitution of flaxseed mucilage for eggs or fat in cupcake formulation represents a deliberate approach to improve both the nutritional and functional characteristics of the final baked product. Flaxseed mucilage possesses inherent emulsifying and water-retention properties, which are critical for replicating the structural and moisture-conserving roles traditionally fulfilled by eggs and fats. In this study, partial replacement at levels of 25–50% preserved essential product qualities such as crumb softness, volume, and moisture balance. These results suggest that flaxseed mucilage can serve as a viable alternative to conventional animal-derived or lipid-rich components, supporting cleaner label formulations without significantly affecting consumer sensory perception.

4. Conclusions

Economic importance of research must be mentioned, with reference to cost of extraction and the cost of laying eggs and fats, in addition to functional importance. It can be concluded that the ultrasound-assisted extraction technique of flaxseed mucilage (FM) provided a higher yield and antioxidant content, along with a shorter extraction time compared to conventional methods. The research demonstrated that FM could effectively replace fat or eggs at a 25% substitution level. Its incorporation improved the sensory attributes of the cupcakes, enhanced moisture content, and reduced both fat and caloric values, supporting the development of healthier baked products and increased total phenolic content (TPC) of cupcakes. Depending on the substitution type, TPC levels increased by 1.90 times (fat replacer), 1.98 times (egg replacer), and 3.92 times (combined fat and egg replacer) relative to the control. This enhancement in phenolic content reflects the bioactive potential of FM and its contribution to the functional value of the final product. The flaxseed mucilage (FM) is reinforced its credibility as a functional food ingredient. Adding flaxseed mucilage to food items like cupcakes may result in many nutritional advantages such as antioxidant properties, healthy fat and other bioactive components. More investigation is required to ascertain the ideal concentration of flaxseed mucilage in various food products for best nutritional advantages as well as to examine the long-term impacts of consuming flaxseed mucilage-enriched food products.

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