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### Improving the Stability of Encapsulated Flaxseed Oil through the Extraction and Utilization of Flaxseed Gum

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#### Abstract

Flax seeds are a type of functional food that offers numerous benefits due to omega-3 fatty acids and flaxseed gum (FSG). FSG is used as an emulsifier and gelling agent in food. During extraction of FSG at 30, 60, and 90°C, both gum and protein yields were observed to increase as temperature increased. However, the ratio of neutral-to-acidic sugar decreased as temperature increased from 30°C to 90°C. The FSG extracted at 90°C had the highest content of total phenols and lignans, resulting in the highest antioxidant activity. The emulsion composed of oil with FSG at a ratio of 0.25:1 exhibited the highest percentage of emulsion stability. The oxidation stability of microencapsulated oil was evaluated by assessing the peroxide value (PV), conjugated dienes (CD), conjugated trienes (CT), and thiobarbituric acid (TBA) during storage under accelerated conditions. The unencapsulated oil showed the highest increases in PV, CD, CT, and TBA compared to encapsulated oils. The encapsulated oil with FSG (0.25:1) had lower contents of PV, CD, CT, and TBA compared to oils with FSG (0.5:1). These results demonstrate the positive effect of using FSG as an ingredient in oil encapsulation for improving oil stability.

Keywords: Flax seed, Flaxseed gum, Lignan, Flaxseed oil, encapsulation.

#### 1. Introduction

Flax seeds, also known as Linum usitatissimum L., come in a variety of physical appearances such as smoothness, shape, length, color, and luster. These seeds are a great source of nutrition due to the presence of health-promoting lignans, alpha-linolenic acid, and soluble flaxseed gum (Taubner et al., 2023). Flaxseed meal is the primary byproduct of flaxseed oil extraction, and is not widely used in food applications because of the presence of antinutritional components such as cyanogen and tannin. However, after the oil extraction process, the flaxseed meal becomes a source of gum that is used as an emulsifier, thickener, and stabilizer in ice cream, yogurt, and creamy cheese. The gum is extracted using water as a solvent (Elsorady, 2016; Akhtar et al., 2019). According to Thierry Hellebois et al. (2021), the extraction method and flaxseed cultivars can affect the yield and physicochemical properties of the flaxseed gum.

Flaxseed gum is a mixture of carbohydrates, proteins, and ash, with ratios of 50-80%, 4-20%, and 3-9%,

respectively. It mainly consists of neutral and acidic polysaccharides, with D-xylose and dL-rhamnose being the dominant neutral and acidic polysaccharides, according to Oomah et al. (1995). Flaxseed gum has both nutritional and functional properties that help reduce the risk of various diseases such as diabetes, cholesterol, heart disease, colorectal cancer, depression, and osteoporosis, as reported by Kaushik et al. (2017) and Vieira et al. (2019). It is a significant co-product of flaxseed meal and can be used as a natural nutritional gum. The amount of flaxseed gum varies from 2% to 10% of the whole flaxseed weight depending on the cultivar and plant growth conditions, according to Li et al. (2007).

Flax seeds are a cost-effective source of phenolic compounds, such as lignans, phenolic acids, flavonoids, and tannins, which have pharmacological and antioxidant properties. These compounds are mainly found in Flaxseed gum, as noted by J.M. Vieira et al. (2019). In fact, flax seeds contain 75-800

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times more of these compounds compared to other seeds, which accounts for about 1-4% of their total weight, according to Alessia Melelli et al. (2022). Due to its high content of lignans, flaxseed is considered to be the richest source of plant lignan (Mazur, 1998).

Flaxseed oil is a type of polyunsaturated oil that is particularly high in  $\alpha$ -linolenic acid, an essential omega-3 fatty acid, which makes up about 57% of the total fatty acids in the oil. It also offers various nutritional benefits and can have a positive impact on health. However, due to the quick oxidation of  $\alpha$ linolenic acid, flaxseed oil can develop an off-flavor, which makes it necessary to protect it during processing, handling, and storage to increase its stability (Chen Cheng, et al., 2021).

Encapsulating oil is a suitable method for protecting food components from oxidation and improving food products (Jeyakumari et al., 2016).

Microencapsulation is a preferred method for this purpose. This is why oil encapsulation is considered to be an appropriate technology that can retard lipid oxidation and expand the range of applications for which oil cannot be used otherwise (Vishnu Anand et al., 2023).

Encapsulation refers to the process of surrounding small particles or droplets with a coating wall or embedding them in a homogeneous or heterogeneous matrix to create microcapsules. This helps to build a barrier between the components in the capsule and the surrounding environment. Over time, numerous technologies have been developed to accurately encapsulate food ingredients.

In the development of microcapsules, selecting the right wall material is a crucial step. This material acts as a protective layer against water, oxygen, and light, and prevents undesirable reactions. Additionally, the stability, water solubility, and emulsifying properties of the microencapsulated emulsion, as well as crosslinking formation during drying, should be evaluated.

This study aimed to extract flaxseed gum and assess its potential to improve the stability of encapsulated flaxseed oil.

## 2. Materials and methods 2.1. Materials

Flaxseed (Giza11) was received from the Agricultural Research Center, Giza, Egypt during the season

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2022-2023. Chemicals and reagents were obtained from Sigma-Aldrich (St.Louis, MO, USA). Louis, MO, US). Louis, US), and El-Gomhoria Co. for Pharmaceutical, Cairo, Egypt.

#### 2.2. Proximate composition

Moisture, protein, oil, fibers, and ash were determined according to AOAC, (2007). Total carbohydrates were determined by difference.

#### 2.3. Extraction of Flaxseed oil

Flax seeds were pressed using hydraulic pressing at room temperature. The extracted oil was filtered, kept in dark brown bottles, and stored at  $-18^{\circ}$ C until analysis.

#### 2.4. Chemical characteristics of Flaxseed oil

Peroxide value (PV), conjugated dienes (CD), conjugated trienes (CT), and thiobarbituric acid (TBA) of the extracted and encapsulating oils were determined according to AOAC, (2007).

#### 2.5. Fatty acids composition of Flaxseed oil

The method of Hanaa M. Soliman et al. (2020) and Hanaa M. Soliman et al. (2022) was used to determine the fatty acids composition of flaxseed oil through esterification. The process involved dissolving 0.1g of oil in 2 ml of heptane, which was then added to a solution of methanolic potassium hydroxide (0.2 ml, 2 N). The mixture was refluxed with stirring for 15 minutes, during which the oil was hydrolyzed to its fatty acids, which were simultaneously esterified to the corresponding methyl esters. The methyl esters were identified using gas chromatography (GC), with a nitrogen flow rate of 0.6 ml/min, air flow rate of 45 ml/min, and hydrogen flow rate of 450 ml/min. The oven was heated to a constant temperature of 195 °C, while the injector and detector temperatures were maintained at 230 and 250 °C, respectively. The concentration of fatty acid methyl esters was detected by comparing their retention times with those of standard fatty acids, and their concentration was determined by the integration of the peak area, which was automatically calculated using an integrator.

#### 2.6. Extraction of Flaxseed gum (FSG)

Flaxseed gum was extracted from flaxseed meal using a modified version of the method described by Kaushik et al. (2017), after oil extraction. The flaxseed meal was soaked in water at a ratio of 1:8 (w/w) at three different temperatures (30, 60, and 90  $^{\circ}$ C) with continuous and gentle stirring over a magnetic plate for 4 hours. Afterward, the soaked meal was filtered, and the filtrate was centrifuged at

4000 rpm for 10 minutes. The gum that precipitated was then vacuum dried at 50  $^{\circ}\mathrm{C}$  and stored at 4  $^{\circ}\mathrm{C}$  for future use.

## 2.7. Effect of extraction temperature on yield and composition of FSG

The yields and compositions of the FSG samples extracted at various temperatures were compared.

#### 2.7.1. Yield determination

Extracted gum yield was calculated as follows:

Yield (%) = (G/S) x 100

where 'G' is the dried gained gum powder weight after extraction from Flax seeds meal and 'S' is the weight of Flaxseed meal.

#### 2.7.2. Proximate composition of FSG

The moisture, protein, and ash content of the FSG samples were determined using the AOAC (2005) method. The measurements represent the mean of triplicate values.

#### 2.8. Neutral and acidic sugar determination

According to Caroline Rondel, et al. (2023), the levels of neutral and acidic sugars were determined using colorimetric assays. The amount of D-xylose and D-galacturonic acid, respectively, per milligram of gum powder was used to express the content of neutral and acidic sugars.

## 2.9. Phenolic compounds extraction and determination

Using Folin-Ciocalteau reagent, total phenol content was calorimetrically determined at 725 nm as described by H.A. Zahran et al., (2018). Where, in a 25 ml volumetric flask, the methanolic solution of gum powder (0.1- 0.3 ml, 5% wt./ v), 20 ml of deionized water, and 0.625 ml of the Folin-Ciocalteau reagent were mixed for 3 min, followed by addition of (2.5 ml ,35%) Na2CO3 solution. the mixture was diluted to a certain volume and left at room temperature for 1 h. Then Through a doublebeam ultraviolet-visible spectrophotometer Hitachi U-3210 (Hitachi, Ltd., Tokyo, Japan) the sample absorbance was measured at 725 nm against a blank. for preparing the calibration curve Gallic acid was used as a standard, and the assay solution ranged from 60 to 140 mg · 25 mL-1.2.10. 2.10. Lignans

#### **Extraction:**

The extraction of lignans was carried out using the method developed by Andrzej Patyra et al. in 2022. Flaxseed meal (200 g) was mixed with 1.2 L of a complex solvent made of ethanol and water (50-100% v/v) at room temperature for 24 hours. The extract was then filtered using a sand core funnel, and

the solvent was evaporated using a rotary evaporator at 40°C and 90 rpm. The resulting syrup was precipitated with 1M NaOH at room temperature for 16 hours, followed by acidification with 1M HCl to pH 6. The solution temperature was then lowered to 15°C, and the salt formed was precipitated by centrifugation at 2000 rpm for 10 minutes. The precipitate was filtered off using a sand core funnel, and the water-soluble polysaccharides and proteins were collected after the freeze-drying process. The weight of lignans was then measured, and their percentage was calculated using the following equation:

Weight of freeze-dried lignans

The acquired ratio of lignans (%) =  $x_{100}$ 

Weight of defatted Flaxseed powder

#### 2.11. DPPH radical scavenging activity

DPPH' solution (2.5 mL , 60  $\mu$ M ) in (0.2 ml) methanol was mixed with 3ml (10 mg / L MeOH) of gum powder, and it was kept in a dark at room temperature for 30 min, where the deep violet DPPH' (1,1-Diphenyl picryl hydrazyl) is reduced to the yellow DPPH (1,1-Diphenyl picryl hydrazine), then absorbance of 200  $\mu$ L of the reaction mixture was determined at 517 nm using portable hyperspectrometer (Spectronic 21D, milton roy Boulder, Colorado, USA). L-Ascorbic acid was used as blank (Hanaa M. Soliman, 2018). The percentage of DPPH radical scavenging activity was calculated using the following formula:

DPPH radical scavenging activity (%) =  $[(A0-A1)/A0] \times 100]$ 

Where A0 was the absorbance of the blank and A1 was the absorbance of the sample or standard. All analyses were performed in triplicate. The data were recorded as mean values.

#### 2.12. Emulsion stability

To test the stability of the FSG solution, we took 25 mL of FSG:oil emulsion (0.5:1, 0.25:1) and placed them in graduated cylinders. These cylinders were then kept in the refrigerator, and we measured the volume of the upper phase at different intervals - 0, 6, 12, and 24 hours, as well as after 7 and 30 days. We calculated the emulsion stability using the following formula:

Separation % = (H1/Ho) \* 100

Here, Ho represents the initial height of the emulsion, and H1 is the height of the upper layer. This method was described by Chen Cheng et al. in 2021.

#### 2.13. Encapsulation procedure

To prepare the samples, 190 g of either A, B, C, D, or E (as listed in Table 1) were mixed with 400 g of 3% Na Alginate and stirred for 30 minutes. The resulting mixture was then dropped onto a 0.5% CaCl<sub>2</sub> solution and left for 2 hours. Afterward, the samples were washed with distilled water and dried using a vacuum oven. The microencapsulation apparatus used for the dropping process was invented by the NRC team and is patented in Egypt under application number 533/2019.

 Table (1): Capsulated sample treatments

Code	Sample				
А	Flaxseed oil				
В	Flaxseed oil: FSG solution (0.5:1)				
С	Flaxseed oil: FSG solution (0.25:1)				
D	Flaxseed oil (lignan 200ppm): FSG solution				
	(0.25:1)				
Е	Flaxseed oil (BHT 200ppm): FSG solution				
	(0.25:1)				

#### 2.14. Encapsulation efficiency

To extract the surface oil, capsules weighing 5 g were mixed with 50 mL of hexane and shaken for 15 seconds at 22°C. The mixture was then filtered, and the unencapsulated oil was collected after vacuum evaporation of hexane. Another set of capsules weighing 5 g was subjected to Soxhlet oil extraction for 4 hours using hexane to measure the percentage of encapsulated oil. The encapsulated oil content was determined after hexane evaporation, as described by Chen Cheng et al. in 2021. The encapsulation efficiency (EE) was calculated using the following formula:

EE = (Total oil - Surface oil) / Total oil x 100

The free oil content was then calculated as a percentage, considering the total oil.

#### 2.15. Assessment of oxidative quality

The capsules were packed in dark glasses to prevent degradation and stored at 37°C in the dark. Quality changes were assessed every 5 days for 20 days by evaluating PV, CD, CT, and TBA for oxidation products of microcapsule oil. Additionally, the effects of encapsulation process and storage conditions were evaluated by subjecting flaxseed oil to the same conditions (37°C in the dark for 20 days). According to Calvo et al. (2011), storing the product under these conditions for 10 days is equivalent to storing it at

room temperature for 30 days in terms of product degradation.

#### 2.16. Statistical analysis

Statistical analysis was expressed as means± SD and was analyzed using a one-way analysis of variance (ANOVA) by SPSS 16.0 software (SSPS Inc., Chicago, IL, USA).

#### 3. Results and discussions

Data in Table (2) shows the chemical compositions of Flaxseed (Giza11), Flaxseed meal after oil extraction, oil characteristics, and fatty acid composition. The results were consistent with Elsorady et al. (2022) for the same variety in different seasons.

#### **Extraction of Flaxseed gum**

According to Table (3), the yield of gum increases as the temperature of extraction increases. This can be attributed to a higher concentration of water-soluble protein and sugar at higher temperatures. Similar results were obtained in studies conducted by Mehtre et al. (2017), Vieira et al. (2019), and Hu et al. (2020), which agree with the findings of Kaushik et al. (2017). The chemical composition of Flaxseed gum extracted at different temperatures was also examined, and the results were consistent with those obtained by Kaushik et al. (2017). The moisture content of Flaxseed gum extracted at 30°C was found to be higher than that extracted at 90°C, which is attributed to the removal of more free water at higher temperatures. The protein content of the gum was found to increase with increasing temperature of extraction, which is consistent with the findings of Thierry Hellebois et al. (2021) and Kaushik et al. (2017). This is likely due to the higher concentration of soluble protein at higher temperatures. Fedeniuk and Biliaderis (1994) reported that Flaxseed gum contained 50-80% carbohydrates, 4-20% protein, and 3-9% ash, and that these variations were primarily due to differences in Flaxseed cultivar, pH, and temperature. Wannerberger et al. (1991) also found similar results. Table (3) shows that neutral sugar is present in greater amounts than acidic sugar. This is consistent with the findings of Caroline Rondel et al. (2023). Kaushik et al. (2017) noted that neutral sugar is associated with lower extraction temperatures of Flaxseed gum, while higher temperatures are associated with acidic sugar. Flaxseed gum typically contains 75% neutral sugar and 25% acidic sugar (Warr et al. 2003). The neutral-to-acidic sugar ratio

was found to decrease with increasing temperature from 30 to 90°C, indicating that higher temperatures are more effective in extracting acidic sugar. This finding is consistent with the results obtained by Kaushik et al. (2017).

Table (2): Chemical com	position of Giza 11	Flax seed, oil characterist	ics, and fatty acid composi	tion
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Chemical composition				
	Flax seed	Flaxseed meal		
Moisture	7.10±0.05	6.54±0.07		
Crude oil	30.54±0.20	8.67±0.05		
Crude protein	21.23±0.11	37.65±0.04		
Ash	4.35±0.03	4.56±0.03		
Carbohydrates	36.78±0.39	42.58±0.19		
Oil characte	eristics			
FFA% (as oleic acid)		0.29±0.03		
Peroxide value (meq O <sub>2</sub> /kg oil)		1.85±0.02		
Conjugated dienes (CD)		1.70±0.00		
Conjugated trienes (CT)		0.22±0.01		
TBA number (mg malonaldehyde/kg oil)		0.36±0.00		
Fatty acid con	position			
C14:0	0.04			
C15:0	0.00			
C15:1	C15:1 0.00			
C16:0	5.47			
C16:1	0.07			
C17:0	0.06			
C17:1	0.04			
C18:0	5.00			
C18:1	16.35			
C18:2	C18:2 15.34			
C18:3 n6	0.23			
C18:3 n3	56.98			
C20:0		0.15		
C20:1	0.14			
C22:0	0.13			
SFA*		10.85		
USFA*	89.15			

\*SFA: Saturated fatty acids; USFA: Unsaturated fatty acids

Data are expressed as mean  $\pm$  SD values given to represent means of three determinations.

Table (3): Effect of extraction temperature on the yield and composition of Flaxseed gum

Extract	Extraction temperature (°C)			
	30	60	90	
Yield %	5.21±0.15 <sup>a</sup>	7.40±0.08 <sup>b</sup>	9.86±0.12 <sup>c</sup>	
	composition of	Flaxseed gum		
Moisture	5.46±0.14 <sup>a</sup>	5.36±0.04 <sup>ab</sup>	5.21±0.10 <sup>a</sup>	
oil	0.4±0.05 <sup>a</sup>	0.51±0.03 <sup>b</sup>	0.62±0.05 °	
protein	4.21±0.09 <sup>a</sup>	7.87±0.08 <sup>b</sup>	9.21±0.07 °	
Ash	0.54±0.03 <sup>a</sup>	0.60±0.03 <sup>a</sup>	0.87±0.05 <sup>b</sup>	
Carbohydrates	89.06±0.86 °	85.57±0.23 <sup>b</sup>	84.09±0.27 <sup>a</sup>	
Neutral sugar g/g	0.40±0.03 <sup>a</sup>	0.49±0.01 <sup>b</sup>	0.57±0.01 °	
Acidic sugar g/g	0.10±0.01 <sup>a</sup>	0.14±0.01 <sup>b</sup>	0.17±0.01 <sup>c</sup>	
Natural/acidic sugar	4.01±0.10 <sup>b</sup>	3.60±0.23 <sup>b</sup>	3.36±0.17 <sup>a</sup>	

The results of the study showed that gum extracted at 90°C had higher levels of total phenols, lignans, and antioxidant activity compared to samples extracted at 30°C and 60°C (as shown in Table 4).

These findings were consistent with those of Vieira et al. (2019). It has been observed that there is a positive correlation between antioxidant activity (DPPH scavenging activity) and phenolic content (Rajurkar

and Hande, 2011; Seçzyk et al., 2017). Aludatt et al. (2016) also reported that the antioxidant activity of phenols extracted through heat treatment is higher than those extracted without heat treatment. This is because phenols have an affinity to form complexes with protein molecules, and at higher temperatures, they are more likely to be extracted in greater quantities by dragging with the protein (Vieira et al., 2019). Lignan is commonly associated and co-extracted with gum polysaccharides of Flax seed, and it provides antioxidant activity. However, the nutritional applications of lignin may have some

drawbacks due to the presence of phytoestrogens as endocrine disruptors (Patisaul and Jefferson, 2010). Based on the results presented in Table 5, it can be concluded that all treatments showed high emulsion stability of 99.7% up to 24 hours. After 7 days, the treatment containing Oil:FSG in the ratio of 0.25:1 had the highest emulsion stability of 99.5%, followed by the treatment containing Oil:FSG in the ratio of 0.5:1 (99.2%), and the treatment containing only FSG (99.1%). The same trend was observed after 30 days. The high emulsion stability observed may be attributed to the good emulsifying properties and high viscosity of FSG.

Table (4): Effect of extraction temperature on phenols, lignin contents and antioxidant activity of Flaxseed gum

Extract	Extraction temperature (°C)				
	30	60	90		
Total phenols (mg GAE/100 g)	13.45±0.35 <sup>a</sup>	15.63±0.15 <sup>b</sup>	18.75±0.09 °		
Lignans (%)	1.92±0.03 <sup>a</sup>	2.43±0.09 <sup>b</sup>	3.06±0.10 <sup>c</sup>		
Antioxidant activity (RSA%)	5.34±0.20 <sup>a</sup>	15.31±0.41 <sup>b</sup>	30.39±0.65 °		

<b>Table (5):</b> Emulsion stability (%) of the treatments						
Treatments	Emulsion stability (%)					
Treatments	0	6 h	12 h	24 h	7 days	30 days
FSG	99.7±0.13 <sup>a</sup>	99.7±0.21 <sup>a</sup>	99.7±0.12 <sup>a</sup>	99.7±0.20 <sup>a</sup>	99.1±0.15 <sup>b</sup>	98.8±0.15 <sup>c</sup>
Oil: FSG (0.5:1)	99.7±0.14 <sup>a</sup>	99.7±0.11 <sup>a</sup>	99.7±0.12 <sup>a</sup>	99.7±0.11 <sup>a</sup>	99.2±0.13 <sup>b</sup>	99.0±0.11 <sup>c</sup>
Oil: FSG (0.25:1)	99.7±0.14 <sup>a</sup>	99.7±0.15 <sup>a</sup>	99.7±0.15 <sup>a</sup>	99.7±0.12 <sup>a</sup>	99.5±0.15 <sup>b</sup>	99.3±0.10 <sup>c</sup>

Fig (1) displays Flaxseed oil capsules. According to Table (6), there were significant differences observed among encapsulated oil, encapsulated oil with FSG (0.5:1), and encapsulated oil with FSG (0.25:1). However, no significant differences were found among encapsulated oil with FSG (0.25:1), encapsulated oil with Lignans (200ppm) with FSG (0.25:1), and encapsulated oil with BHT (200ppm) with FSG (0.25:1). The encapsulated oil had the lowest efficiency of 95.61%, while the highest efficiency of about 97% was observed in oil treatments with FSG (0.25:1). Tonon et al. (2011) reported that the lowest encapsulation efficiency of Flaxseed oil was observed for whey protein, while the highest efficiency was observed for modified starch and Arabic gum.

Table 7 presents the fatty acid composition of Flaxseed oil (Giza 11) and encapsulated oils. The most common category was PUSFA, followed by MUSFA and SFA. These findings are consistent with those of Tavarini et al. (2019), Elsorady et al. (2022), and indicate that there were slight differences in fatty acid compositions between encapsulated and un-

encapsulated oil samples. This may be attributed to FSG and antioxidants.



**Figure (1):** Capsulated samples, A: Encapsulated oil; B: Encapsulated oil with FSG (0.5:1); C: Encapsulated oil with FSG (0.25:1); D: Encapsulated oil with Lignans (200ppm) with FSG(0.25:1); E: Encapsulated oil with BHT(200ppm) with FSG(0.25:1)

#### Microcapsule stability under acceleration test

During storage under acceleration conditions, the oxidation stability of microencapsulated oil was evaluated by assessing the primary and secondary products of the oxidation process, namely PV, CD, CT, and TBA. Figure 2 illustrates the PV (meqO2/kg oil) for unencapsulated and encapsulated oils during storage at 37°C. Generally, the PVs increased with increasing storage time at 37°C up to 20 days. The unencapsulated oil had the highest PV (20.44 meqO2/kg oil) after storage at 37°C for 10 days, while the encapsulated oils had the lowest PV (17.24-20.65 meqO2/kg oil) after storage at 37°C for 20 days. The data also showed that the PV of encapsulated oil with FSG (0.25:1) was lower than that of encapsulated oil with FSG (0.5:1) during storage at 37°C for 20 days. Additionally, the encapsulated oil with FSG (0.25:1) with 200ppm BHT had a lower PV than that of encapsulated oil with FSG (0.25:1) with 200 ppm lignan during storage at 37°C for 20 days. Calvo et al. (2010) indicated that lower encapsulation efficiency results in increased susceptibility to oxidation. Low encapsulation efficiency means that a high amount of oil is available for oxidation on the particle surface (Tonon et al., 2012).

The primary product of oxidation is the measurement of Conjugated diene (CD) at 232 nm, as stated by Elsorady and Abdelaziz in 2011. Figure 3 shows that during a 20-day storage period at 37°C, the content of CD increased. The unencapsulated oil showed a higher increase in CD than the capsulated oils. The CD content was lower in encapsulated oil with FSG (0.25:1) than that with FSG (0.5:1). BHT was found to be more effective in delaying CD formation and was a higher antioxidant than lignan.

Figures 4 and 5 illustrate the changes in CT and TBA measurements of both encapsulated and unencapsulated oils during 20 days of storage at  $37^{\circ}$ C. TBA test was used to measure the levels of malonaldehyde, a secondary oxidation product. The results indicate that the trend observed for CD and PV was consistent with CT and TBA values. As storage time increased at  $37^{\circ}$ C, the CT and TBA values of both encapsulated and unencapsulated oils also increased. Among the oils tested, unencapsulated oils had the highest CT and TBA values, while encapsulated oil with BHT with FSG (0.25:1) had the lowest CT and TBA values.

Additionally, CT and TBA values of encapsulated oil with FSG (0.25:1) were lower than those with FSG (0.5:1). The study reported that the secondary product

of oxidation of encapsulated buttermilk algal oil increased with storage time.



**Figure (2):** Effect of storage time of un-encapsulated and encapsulated Flaxseed oils at 37°C on PVs. [1: Un-encapsulated oil; 2: Encapsulated oil; 3: Encapsulated oil with FSG (0.5:1); 4: Encapsulated oil with FSG (0.25:1); 5: Encapsulated oil with 200 ppm Lignans with FSG (0.25:1); 6: Encapsulated oil with 200 ppm BHT with FSG (0.25:1)]



Figure (3): Effect of storage time of un-encapsulated and encapsulated Flaxseed oils at  $37^{\circ}$ C on CDs. [1: Un-encapsulated oil; 2: Encapsulated oil; 3: Encapsulated oil with FSG (0.5:1); 4: Encapsulated oil with FSG (0.25:1); 5: Encapsulated oil with 200 ppm Lignans with FSG (0.25:1); 6: Encapsulated oil with 200 ppm BHT with FSG (0.25:1)].



**Figure (4):** Effect of storage time of unencapsulated and encapsulated Flaxseed oils at 37°C on CTs. [1: Un-encapsulated oil; 2: Encapsulated oil; 3: Encapsulated oil with FSG (0.5:1); 4: Encapsulated oil with FSG (0.25:1); 5: Encapsulated oil with 200 ppm Lignans with FSG (0.25:1); 6: Encapsulated oil with 200 ppm BHT with FSG (0.25:1)]

Treatments	Encapsulation efficiency (%)
Encapsulated oil	95.61±0.17 <sup>a</sup>
Encapsulated oil with FSG (0.5:1)	96.10±0.11 <sup>b</sup>
Encapsulated oil with FSG (0.25:1)	97.21±0.07 <sup>c</sup>
Encapsulated oil with Lignans (200ppm) with FSG (0.25:1)	97.23±0.07 °
Encapsulated oil with BHT (200ppm) with FSG (0.25:1)	97.24±0.11 <sup>c</sup>

 Table (6): The encapsulation efficiency (%) of different encapsulation treatments:

**Table (7):** Fatty acid composition of flaxseed oil and encapsulated oil samples

		Capsulated flaxseed ons						
	Florecod		Encapsulated	Encapsulated	Encapsulated	Encapsulated oil		
Fatty acids	oil	Encapsulated	oil with	oil with	oil with Lignans	with		
	011	oil	FSG(0.5:1)	FSG(0.25:1)	(200ppm) with	BHT(200ppm)		
					FSG(0.25:1)	with FSG(0.25:1)		
C14:0	0.04	0.15	0.05	0.03	0.05	0.04		
C16:0	5.47	5.52	5.91	5.08	5.62	5.18		
C16:1	0.07	0.22	0.08	0.06	0.10	0.07		
C17:0	0.06	0.08	0.07	0.07	0.14	0.06		
C17:1	0.04	0.05	0.03	0.03	0.11	0.03		
C18:0	5.00	5.63	5.59	5.63	5.53	5.29		
C18:1	16.35	18.04	17.45	17.97	16.26	17.37		
C18:2	15.34	15.69	16.02	15.89	15.21	15.75		
C18:3 n6	0.23	0.21	0.17	0.19	0.48	0.19		
C18:3 n3	56.98	53.67	54.20	54.54	55.40	55.62		
C20:0	0.15	0.35	0.14	0.18	0.53	0.15		
C20:1	0.14	0.25	0.17	0.17	0.45	0.12		
C22:0	0.13	0.14	0.12	0.16	0.12	0.13		
SFA <sup>*</sup>	10.85	11.87	11.88	11.15	11.99	10.85		
USFA <sup>*</sup>	89.15	88.13	88.12	88.85	88.01	89.15		
MUSFA	16.60	18.56	17.73	18.23	16.92	17.59		
PUSFA	72.55	69.57	70.39	70.62	71.09	71.56		

\*SFA: Saturated fatty acid; USFA: Unsaturated fatty acid, MUSFA: Monounsaturated fatty acid; PUSFA: Polyunsaturated fatty acid



Figure (5): Effect of storage time of un-encapsulated and encapsulated Flaxseed oils at 37°C on TBA. [1: Un-encapsulated oil; 2: Encapsulated oil; 3: Encapsulated oil with FSG (0.5:1); 4: Encapsulated oil with FSG (0.25:1); 5: Encapsulated oil with 200 ppm Lignans with FSG (0.25:1); 6: Encapsulated oil with 200 ppm BHT with FSG (0.25:1)]

#### 4. Conclusions

Based on our analysis, we can confidently say that the most optimal temperature for FSG extraction is 90 °C. This temperature provides a high yield of FSG and retains a significant amount of bioactive omponents. In addition, the use of FSG as an ingredient in oil encapsulation has a positive effect on the shelf life of the product.

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#### Authors' contributions

All authors contributed equally in all parts of this study.

**Ethics approval and consent to participate** Not applicable.

#### **Consent for publication**

Not applicable.

#### **Competing interests**

The authors declare that they have no competing interests.

#### 5. References

- Akhtar, M.N., Mushtaq, Z., Ahmad, N., Khan, M.K., Ahmad, M.H., Hussain, A.I. and Imran, M. (2019). Optimal Ultrasound-Assisted Process Extraction, Characterization, and Functional Product Development from Flaxseed Meal Derived Polysaccharide Gum. Processes, 7, 189; doi:10.3390/pr7040189
- Alessia Melelli, Frédéric Jamme, Johnny Beaugrand, Alain Bourmaud. (2022).Evolution of the ultrastructure and polysaccharide composition of flax fibres over time: When history meets science. Carbohydrate Polymers, 291, 119584.
- Aludatt, M. H., Rababah, T., Alhamad, M. N., Gammoh, S., Ereifej, K., Kubow, S., *et al.* (2016). Characterization and antioxidant activities of phenolic interactions identified in byproducts of soybean and Flaxseed protein isolation. Food Hydrocolloids, 61, 119–127.
- Andrzej Patyra, Małgorzata Kołtun-Jasion, Oktawia Jakubiak, Anna Karolina Kiss. (2022). Extraction Techniques and Analytical Methods for Isolation and Characterization of Lignans Andrzej. Plants 2022, 11, 2323.
- AOAC. Official Methods of Analysis of the Association of Official Analytical Chemists 18th edn. (AOAC International, 2005).
- Calvo P, Castano AL, Hernandez T and Gonzalez-Gomez D, (2011). Effects of microcapsule constitution into the quality of microencapsulated

walnut oil. Eur J Lipid Sci Technol 113, 1273-1280.

- Calvo P, Hernandez T, Lozano, M. and Gonzalez-Gomez, D. (2010). Microencapsulation of extravirgin olive oil by spray-drying: Influence of wall material and olive quality. Eur J Lipid Sci Technol 112, 852–858.
- Caroline Rondel, Claire-Emmanuelle Marcato-Romain, Elisabeth Girbal-Neuhauser. (2023).
   Development and validation of a colorimetric assay for simultaneous quantification of neutral and uronic sugars. water research 47,2901 – 2908.
- Chen Cheng, Xiao Yu, Fenghong Huang, Dengfeng Peng, Hongjian Chen, Yashu Chen, Qingde Huang, Qianchun Deng. (2021). Effect of different structural Flaxseed lignans on the stability of Flaxseed oil-in-water emulsion: An interfacial perspective. Food Chemistry, 357, 129522.
- Cui, W., Mazza, G. and Biliaderis, C.G. (1994). Chemical Structure, Molecular Size Distributions, and Rheological Properties of Flaxseed Gum. *Journal of Agricultural and Food Chemistry* 42 (9), 1891–1895.
- Elsorady M.E.I., El-Borhamy A.M.A. and Barakat E.H.A. (2022). Evaluation of new Egyptian Flaxseed genotypes and pasta fortified with Flax seeds. Acta Scientiarum.Technology, 44, e57014. Doi: 10.4025/actascitechnol.v44i1.57014
- Elsorady, M. E. I. and Abdl Aziz, M.A. (2011). Antioxidant Activities of Thyme Oils Extracted from Organic and Chemical Treated Plants and their Influence on Sunflower Oil Oxidation. Egypt. J. Agric. Res., 87 (1), 109-125.
- Elsorady, M.E. (2016). Utilization of hydrocolloids (Flaxseed cake and Sesame cake) on oil decrease absorption in potato strips during deep frying. J. Biol. Chem. Environ. Sci., 11(4), 63-83.
- Fedeniuk, R.W. and Biliaderis, C.G. (1994). Composition and Physicochemical Properties of Linseed (Linum Usitatissimum L.) Mucilage. Journal of Agricultural and Food Chemistry, 42, 240–240.
- H.A. Zahran and H.M. Soliman. (2018). UPLC-Q-TOF/MS Screening of Bio-Active Compounds Extracted from Olive Mill Solid Wastes and their Effect on Oxidative Stability of Purslane Seed Oil, Current Science International, 7(2): 307-319.
- Hanaa M. Soliman & Hamdy A. Zahran. (2022). Synthesis of a new hydrophobic coating film from

stearic acid of buffalo fat, Scientific Reports 12, 18465.

- Hanaa M. Soliman & Y. El- Shattory. (2020). Isolation of Pure Individual Fatty Acids from Chicken Skin Using Supercritical CO2 Extractor or Cooling Centrifuge, Journal of Oleo Science. 69, (8) 859-864.
- Hanaa M. Soliman. (2023). 'Preparation of a Regioselective Quercetin-3-palmitate and its using for Boosting Cooking Oil Stability, Journal of Oleo Science. 72, (2).
- Hu, Y., Shim, Y.Y. and Reaney, M.J.T. (2020). Flaxseed Gum Solution Functional Properties. Foods 2020, 9, 681; doi:10.3390/foods9050681
- J.M. Vieiraa, R.A. Mantovania , M.F.J. Raposob , M.A. Coimbrab , A.A. Vicentec , R.L. Cunhaa. (2019). Effect of extraction temperature on rheological behavior and antioxidant capacity of Flaxseed gum. Carbohydrate Polymers, 213, 217-227.
- Jeyakumari, A., Janarthanan, G., Chouksey, M. K., and Venkateshwarlu, G. (2016). Effect of fish oil encapsulates incorporation on the physicochemical and sensory properties of cookies. Journal of Food Science and Technology, 53(1), 856–863. https://doi.org/ 10.1007/s13197-015-1981-2
- Kaushik, P., Dowling, K., Adhikari, R., Barrow, C.J. and Adhikari, B. (2017). Effect of extraction temperature on composition, structure and functional properties of Flaxseed gum. Food Chemistry 215 (2017) 333–340
- Li, L., Kou, Z., Zhu, J., and Wang, Y. (2007). Isolation of secoisolariciresinol diglucoside from Flaxseed with macroporous adsorption resin. China Oils and Fats, 32(8), 52-55.
- Mazur, W. (1998). 11 Phytoestrogen content in foods. Bailliere's Clinical Endocrinology and Metabolism, 12(4), 729-742.
- Mehtre, A.S., Syed, H.M. and Agrawal, R.S. (2017). Extraction and chemical composition of Flaxseed gum (mucilage)from different Flaxseed varieties. The Bioscan, 12(1), 47-49.
- Oomah, B. D., Kenaschuk, E. O., Cui, W. and Mazza, G. (1995). Variation in the compositionof water-soluble polysaccharides in Flax seed. J. Agricultural and Food Chemistry. 43, 1484-1488.
- Patisaul, H. B. and Jefferson, W. (2010). The pros and cons of phytoestrogens. Frontiers in Neuroendocrinology, 31(4), 400–419.

- Rajurkar, N.S. and Hande, S.M. (2011). Estimation of phytochemical content and antioxidant activity of some selected traditional Indian medicinal plants. Indian Journal of Pharmaceutical Sciences, 73(2), 146–151.
- Seçzyk, L., Swieca, M., Dziki, D., Anders, A., and Gawlik-Dziki, U. (2017). *Antioxidants*, nutritional and functional characteristics of wheat bread enriched with ground Flaxseed hulls. Food Chemistry, 214, 32–38.
- T. Taubner, M. Skr<sup>\*</sup>ivan, M. Englmaierová, L. Malá (2023). Effects of hemp seed and Flaxseed on enzyme activity in the broiler chicken digestive tract. Animal, 17, 100765.
- Tavarini, S., Castagna, A., Conte, G., Foschi, L., Sanmartin, C., Incrocci, L., Ranieri, A., Serra, A. and Angelini, L.G. (2019). Evaluation of Chemical Composition of Two Linseed Varieties as Sources of Health-Beneficial Substances. Molecules, 24, 3729; doi:10.3390/molecules24203729
- Thierry Hellebois, Jennyfer Fortuin, Xuan Xu, Alexa nderS. Shaplov, Claire Gaiani, Christos Soukoulis . (2021). Structure conformation, physicochemical and rheological properties of Flaxseed gums extracted under alkaline and acidic conditions. International Journal of Biological Macromolecules, 192, 1217-1230.
- Tonon, R.V., Grosso, C.R. and Hubinger, M.D. (2011). Influence of emulsion composition and inlet air temperature on the microencapsulation of Flaxseed oil by spray drying. Food Res Int., 44(1):282–9. https:// doi. org/ 10. 1016/j. foodr es. 2010. 10. 018.
- Tonon, R.V., Pedro, R.B., Grosso, C.R. and Hubinger, M.D. (2012). Microencapsulation of Flaxseed oil by spray drying: effect of oil load and type of wall material. Drying Tech., 30, 1491– 501. https:// doi. org/ 10. 1080/ 07373 937. 2012. 696227.
- Vieira, J.M., Mantovani, R.A., Raposo, M.F.J., Coimbra, M.A., Vicente, A.A. and Cunha, R.L. (2019). Effect of extraction temperature on rheological behavior and antioxidant capacity of Flaxseed gum. Carbohydrate Polymers, 213, 217– 227
- Vishnu Anand, Vikono Ksh, Abhijit Kar, Eldho Varghese, Sujata Vasudev, Charanjit Kaur. (2023) Encapsulation efficiency and fatty acid analysis of chia seed oil microencapsulated by freeze-drying

using combinations of wall material. Food Chemistry, 430, 136960.

- Wannerberger, K., T. Nylander, and M. Nyman. (1991). Rheological and Chemical Properties of Mucilage in Different Varieties from Linseed (Linum Usitatissimum). Acta Agriculturae Scandinavica 41 (3): 311–319.
- Warr, J., Michaud, P., Picton, L, Muller, G., Courtois, B., Ralainirina, R. and Courtois, J. (2003). Large-Scale Purification of Water-Soluble Polysaccharides from Flaxseed Mucilage, and Isolation of a New Anionic Polymer. *Chromatographia* 58 (5–6), 331–335.
- Zhang Y, Pang X, Zhang S, Liu L, Ma C, Lu J, Lyu J. (2020). Buttermilk as a wall material for microencapsulation of omega-3 oils by spray drying. LWT., 127, 109320. https:// doi. org/ 10.