

# Extraction, Composition and Physicochemical Properties of Flaxseed Mucilage

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## ABSTRACT

Flaxseed mucilage (*linum usitatissimum* L.) of Blinka variety was extracted using water (1: 20 w/v) at 25-100°C. The mucilage was precipitated from the extract with 80% ethanol in water (1:4 v/v). The proximate composition, sugar composition, and functional properties in terms of solubility, foaming capacity and stability, viscosity and microstructure were studied. Results revealed that extraction with water at 25°C yielded only 3.0 to 5.2% mucilage after 8 hours of extraction, while the extractions with boiling water yielded 8 % mucilage over the same time. However, the finished product in the latter case was considerably darker than the extracted with water at room temperature (25°C). Therefore, to reduce browning and increase the yield of mucilage, the extraction was carried out at room temperature but starting with boiling water. Using this procedure, over 90% of the mucilage could be extracted within 4 hr. The proximate composition of mucilage extracted with boiling water at 100°C showed higher crude oil, protein, and ash contents than that of samples extracted with water at room temperature. Data also showed that the temperature of the extraction had influenced the sugar composition of the polymers. Data revealed that the solubility of 0.5% solution of flaxseed mucilage was between 70 - 90% and indicated that it could be readily solubilized at concentrations up to 0.5%. Flaxseed mucilage gave foam values about 75% of those of ovalbumin and had similar time-dependent stability. The viscosity of flaxseed mucilage decreased as the shear rate increased with all concentrations used. At low concentrations the flow curves tended to be a Newtonian behavior while at concentrations above 0.3% the solutions exhibited shear thinning with increasing shear rate, which is typical of polymeric solutions. The solubility, foam stability viscosity and microstructure data suggested that flaxseed mucilage could be used as a substitute for gum Arabic in food formulations.

## INTRODUCTION

Flaxseed (*Linum usitatissimum* L.) is extensively used for the commercial productions of both fiber and oil. Flaxseed contains a seed coat (hull) with a thin endosperm, an embryo axis and two cotyledons comprising 36%, 4% and 55%, respectively of the total seed weight (Bhatty and Cherdkiatgumchai, 1990). Flaxseed hull consists of five distinct layers with the outer most surface layer (epiderm) incorporating a mucilaginous carbohydrate material (Freeman, 1995). The mucilage accounts for

approximately 8% of the flaxseed weight (Mazza and Biliaderis, 1989). The true hull (or spermoderm) is covered on the outside by epiderm containing mucilage (58%) (BeMiller *et al.*, 1993) and on the inside by the endosperm.

Flax mucilage is a heterogenic polysaccharide and contributes largely to the soluble fiber fraction of flaxseed which is suggested to have a hypoglycaemic effect in humans (Cunnane *et al.*, 1993). Flaxseed mucilage can be easily extracted with water either from the fiber portion or from the whole seed (Cui, *et al.*, 1994; Warrand *et al.*, 2003). The extraction yield, the level of protein, and the physicochemical properties of mucilage are a function of temperature, pH, ratio of water to seeds, duration of the extraction and variety of the raw material (Cui & Mazza, 1996; Fedeniuk and Biliaderis, 1994; Oomah *et al.*, 1995). So far, the majority of studies on flaxseed mucilages focused on the optimization of mucilage extractions (Oomah *et al.*, 2001), the characterization of the physicochemical properties of the mucilage in solution (Goh *et al.*, 2006; Oomah *et al.*, 1995; Stewart and Mazza, 2000; Warrand *et al.*, 2005).

Flaxseed mucilages are commonly employed in the cosmetic industry as texturing agents; however, in the food industry, their application has not yet been extensively examined. Polysaccharides extracted from flaxseed have shown promise as a novel food ingredient, however, very little is understood on its effect when added to food emulsions (Alix *et al.*, 2009). Very limited data are available on flaxseed mucilage of varieties grown in Egypt. Therefore, the aim of this research was to study the extraction, composition, and the physicochemical properties of mucilage from flaxseed varieties grown in Egypt.

## **MATERIALS AND METHODS**

### **Flaxseeds**

Three flaxseed (*linum usitatissimum* L.) variety: Blinka, was obtained from Fiber Crops Institute (Sakha, kafr El-Shekh), Agriculture Research Center, Egypt during season 2007/2008. Flaxseed were cleaned and kept in paper bags at room temperature until further analysis.

### **Chemicals**

Solvent used in this investigation were purchased from El-Gomhouria Pharmaceutical Company and EL-Nasr Pharmaceutical Company, Egypt and the fine chemicals were purchased from Sigma (St Louis, MO, USA).

## Methods

### Extraction of mucilage

Flaxseed mucilage was extracted according to the method described by (Bhatty, 1993). Flaxseeds were mixed with water (1:20 w/v) and stirred for (0.5-8 hr) at 25-100°C. The extract was separated by filtration using glass wool. The mucilage solution was then concentrated on a rotary evaporator at 40°C. The mucilage was precipitated from the extract with 80% ethanol in water (1:4 v/v). After allowing standing for 1 hr at 4°C, the precipitate was collected by centrifugation at 6000 rpm for 35 min, homogenized in water and freeze-dried.

### Proximate composition of flaxseed mucilage

Moisture content was determined by drying the samples at 105°C, total nitrogen, crude fat content, ash content were determined according to the methods as described by the AOAC (1990). Total carbohydrate of flaxseed mucilage content was determined by difference.

The sugar content of the extracted mucilage was studied. The concentrations of the total sugars and of the acidic sugars were determined by micro-scale colorimetric assays. The total quantities of carbohydrate were determined by a method described by Dubois using the measurement of the absorbance at 485 nm after reaction with phenol in the presence of sulphuric acid.

The sugar composition of extracted mucilage was also investigated using a (GC) after hydrolysis of the polysaccharides and trimethylsilylation of the sugars. The samples (0.5–1 mg) were first dissolved in trifluoroacetic acid (2 N), warmed at 110°C for 2 h and dried. Then, they were methanolysed in 1 M anhydride acid in methanol (16 h, 80°C) using inositol as an internal standard. Separation and analysis of trimethylsilylated derivatives were performed using GC on capillary columns (DB 225; J.W. Instruments) with nitrogen as a carrier gas and an air–hydrogen mixture as fuel.

### Functional properties

Foaming capacity and stability of mucilage was studied according to the methods of Sathe and Salunkhe (1981). A sample (1 g) was whipped with 100 ml distilled water for 5 min in a blender at speed setting "HI" and was poured into 250 ml cylinder. The total volume was noted at time intervals of

0.0, 0.5, 1.0, 2.0, 3.0, 4.0, 5.0; 6.0 hr. Specific volume and volume increase (%) were calculated according to the following equations:

$$\text{Specific volume} = \frac{\text{vol after whipping (ml)}}{\text{wt. after whipping}}$$

$$\text{Volume increase} = \frac{\text{vol after whipping} - \text{vol before whipping (ml)}}{\text{vol. before whipping}} \times 100$$

**Viscosity measurements** were carried out using aqueous solutions of 0.3, 0.5, and 0.7 % (w/ v) mucilage (pH 6.5) as described by Mazza and Biliaderis, (1989). Mucilage was solubilized by stirring in hot distilled water. Thick dispersions were centrifuged at 5000 rpm for 15 min to remove entrapped air bubbles. The viscosity was determined using the Brookfield digital viscometer (model DV- E) at room temperature.

**Solubility** of flaxseed mucilage was determined using a modification of the procedure reported by Mazza and Biliaderis, (1989) The mucilage was dispersed in distilled water (0.5% w/v) and the resultant slurry heated under stirring at the desired temperature for 30 min in a temperature-controlled water bath, cooled at 10°C, and centrifuged at 10°C in a Hettich centrifuge, model Rota fix32. for 10 min at 5000 rpm and 10 additional min at 10,000rpm. Aliquots (10 mL) of the supernatant were dried to a constant weight at 105°C to determine the percent solubilization of the mucilage. It should be noted that this procedure, which was routinely used to measure solubility of starches, does not give results on the effect of temperature on solubility but on solubilization of the gums. Thus, solubility was determined only at 10°C.

### Scanning electron microscopy

The (mucilage) was individually observed by SEM using a (Jeol JSM 6360 LA) microscope. Beforehand, samples were broken under liquid nitrogen at -190°C and then covered with a gold layer. The sections were observed to determine the homogeneity of mucilage.

## RESULTS AND DISCUSSION

### Extraction of the flaxseed mucilage

Effects of extraction time and temperature on mucilage yield from belinka flaxseed variety are shown in Table (1). Data revealed that

extraction with water at 25°C yielded only 3.0 to 5.2% mucilage after 8 hours of extraction, while the extractions with boiling water yielded 8 % mucilage over the same time. However, the finished product in the latter case was considerably darker than the extracted with water at room temperature (25°C). Therefore, to reduce browning and increase the yield of mucilage, the extraction was carried out at room temperature but starting with boiling water. Using this procedure, over 90% of the mucilage could be extracted within 4 hr. Similar results were reported by Mazza and Biliaderis (1989) using *linott* flaxseed variety. Mazza and Biliaderis (1989), Bhatta and Cherdkiatgumchai (1990) and Fedeniuk and Biliaderis (1994) reported that water is the most suitable extraction medium for flaxseed mucilage intended for further studies.

**Table (1): Effect of time and temperature on extraction yield of mucilage from belinka flaxseed variety**

Extraction time (hr)	Mucilage concentration (g/100gm seed)		
	25°C	100-25°C <sup>a</sup>	100°C
1	3.00	3.90	4.80
2	4.60	6.70	7.00
4	4.90	7.00	7.30
6	5.20	7.20	8.00
8	5.20	7.20	8.00

### Proximate composition of flaxseed Mucilage

Table (2) shows the proximate composition of mucilage from belinka flaxseed. Data revealed that mucilage samples extracted with boiling water at 100°C showed higher crude oil (0.7%); protein (14.0%) and ash contents (12.0%) than that of samples extracted with water at room temperature (25°C), i.e., 0.45; 3.80; and 11.5 % (on dry basis), respectively. Mazza and Biliaderis (1989) reported that the protein content of mucilage samples of *linott* flaxseed variety extracted with boiling water was higher than that of samples extracted with water at room temperature (4.6% dry basis). However, ash, fat, and mineral contents were very similar.

**Table (2): Proximate and sugar composition of mucilage from belinka flaxseed variety**

Component	Flaxseed mucilage extracted at		
	25°C	100-25°C <sup>a</sup>	100 °C
Moisture	7.00	3.90	6.20
Crude oil	0.45	0.30	0.70
Crude protein	3.80	12.00	14.00
Ash	11.50	11.00	12.00
Carbohydrate (by difference )	84.25	76.70	73.30
Uronic acid %	22.10	23.40	15.30
Rhamnose	20.30	15.40	9.30
Galactose	15.40	19.20	22.10
Arabinose	6.10	9.20	10.20
Xylose	34.50	30.20	28.60
Glucose	1.60	2.60	14.50

(a) Extraction performed initially with boiling water at 100°C.

The extractions at 25°C and at 100 to 25°C gave viscous and sticky mucilage, which became fibrous, compact, and easy to recover after precipitation in ethanol. The mucilage extracted, however, at 100°C was jelly and slightly degraded and showing some browning due to the presence of tannin). After precipitation in ethanol, it became poorly soluble in water except in boiling water, possibly due to the presence of starch. The total carbohydrate content as well as the polymer fraction (obtained after precipitation with ethanol) decreased with increasing temperatures. It is suggested that starch was progressively degraded due to the induction of amylase activity so as starch oligomers were released but not precipitated with ethanol. Reactions such as galactosidase activity might also occur as the amount of galactose is higher in mucilage extracted at 25°C before ethanol precipitation (Alix *et al.*, 2008). On the other hand, as previously reported by (Alix *et al.*, 2008), numerous proteins were solubilised at high temperature but might be insoluble after alcohol precipitation. Two main types of polymer have been identified by Alix, *et al.* (2008), and were acidic pectin-like molecules called rhamnogalacturans and neutral arabinoxylans. The neutral polysaccharide has a larger molecular size and exhibits shear thinning flow behavior in aqueous solutions above 1% (w/w), whereas the acidic polysaccharide has lower molecular size polymers and exhibits Newtonian-like flow behavior even at a much higher concentration.

Flaxseed mucilage has potential industrial use because its emulsifying properties were better than those of Tween 80, gum Arabic and gum tragacanth. Flaxseed hydrocolloidal gum has previously been removed by aqueous extraction (Suşheelamma, 1987; Fedeniuk and Biliaderis, 1994); however, the wet process is relatively expensive since it involves multiple steps, including drying. Attempts have also been made to remove flaxseed hydrocolloidal mucilage with dry dehulling of seeds by grinding and sieving to obtain low and high protein products.

### Sugar composition

Table (2) shows sugar composition of mucilage from belinka flaxseed variety. Data showed that the temperature of the extraction influenced the sugar composition of the polymers although all fractions contained the two mucilage types. The sugars specific to the rhamnogalacturonan backbone (galacturonic acid and rhamnose) were the most abundant at the low temperature, i.e. in mucilage extracted at 25°C, the rhamnose content was 20.3% and that extracted from 100 to 25°C was 15.4% and decreased to 9.3% when extracted at 100°C. In these mucilages, the ratio of galactose (Gal) to rhamnose (Rha) increased from 0.75 to nearly 1.3. at 100-25°C. The latter being similar to that reported by Alix *et al.* (2008) and slightly higher than those reported in the literature (e.g. 0.5 in], 0.6 in( Warrand, *et al*,2003), up to 0.8–0.9. Besides, this ratio increased up to 2.5 at 100°C. Arabinose (Ara) content, however, increased as the temperature of extraction increased. Its content increased from 6.1 at 25°C to 9.2 at 100 to 25°C and finally increased to 10.2 at 100°C, suggesting that there might be some release of arabinogalactan (AG) due to the high temperature. Two types of AG have to be considered: either pectic AG-I consisting of a  $\beta$ -1-4 galactan backbone with  $\alpha$ -1-5 arabinan side chains or AG-II consisting of complex carbohydrate moieties (similar to gum arabic) branched onto a protein backbone, the whole structure designated as arabinogalactan protein (AGP) (Alix *et al.*, (2008).

Contrary, the percentage of xylose (Xyl), the sugar specific to the  $\beta$ -1-4 xylan backbone present in the mucilage decreased as the temperature of extraction increased, and ranged from 34.5 to 28.6% for mucilage fractions, which was lower than in data reported from other varieties such as yellow linseed of Laboulet Est. (Airaines, France) ( Warrand,*et al*,2003). The ratio of (Ara) to (Xyl) increased from 0.18 to 0.35 with the increasing the extraction temperature. It was of the same order as the previously reported values comprised between 0.2 and 0.56 According to Warrand, *etal* ,2005. arabinoxylans contain populations of polysaccharides with

different molecular weight (about 5,000,000 g /mol (less than 10%), 1,000,000 g/ mol (40%), 200,000 g/ mol (50%)). They also varied in the amount of galactose present in the branched side chains. Glucose content also increased as the extraction temperature increased from 1.6 at 25°C to 2.6 at 100-25°C then 14.5 % for mucilage extracted at 100°C.

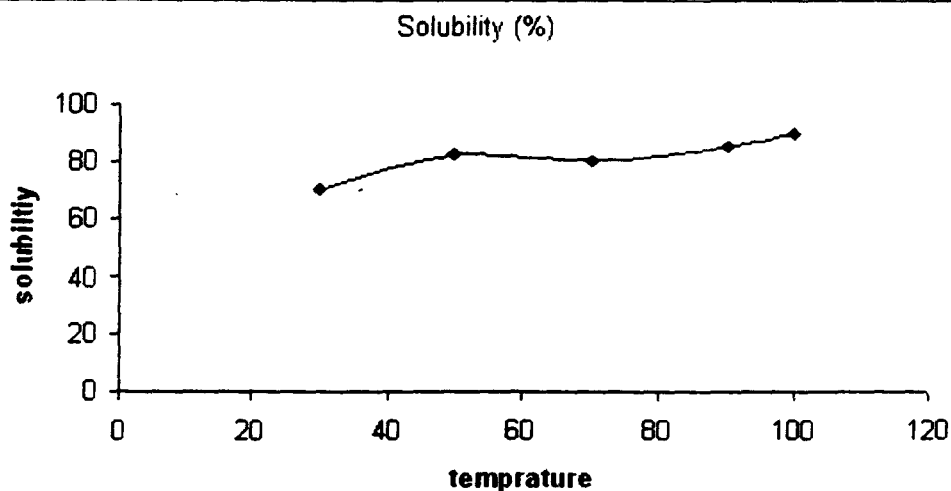
The level of galacturonic acid in flaxseed mucilage reflected the relative amount of acidic polysaccharides in the mucilage. Previous findings have indicated that mucilage high in acidic polysaccharide exhibited typical Newtonian-like flow behaviour in aqueous solutions (Cui, *et al.*, 1994). Thus, mucilage containing a higher amount of acidic polysaccharides exhibit weaker rheological properties. In contrast, the level of xylose in the mucilage reflected the relative amount of neutral polysaccharides, which enhances the rheological properties of the mucilage by increasing the characters of shear thinning and weak-gel properties (Cui *et al.*, 1994)

## **Functional properties of mucilage**

### **Solubility**

Solubility of 0.5% aqueous solutions of flaxseed at 30-100°C is shown in Figure (1). Data revealed that the solubility of 0.5% solution of flaxseed mucilage was between 70 and 90% and indicated that it could be readily solubilized at concentrations up to 0.5% (w/v). This was much higher than the concentration range of 0.1-0.2% reported by BeMiller (1973) and exactly similar to those reported by Mazza and Biliaderis (1989). Data also revealed that temperatures at which the gums were solubilized had little effect on solubility at 10 and 20°C.

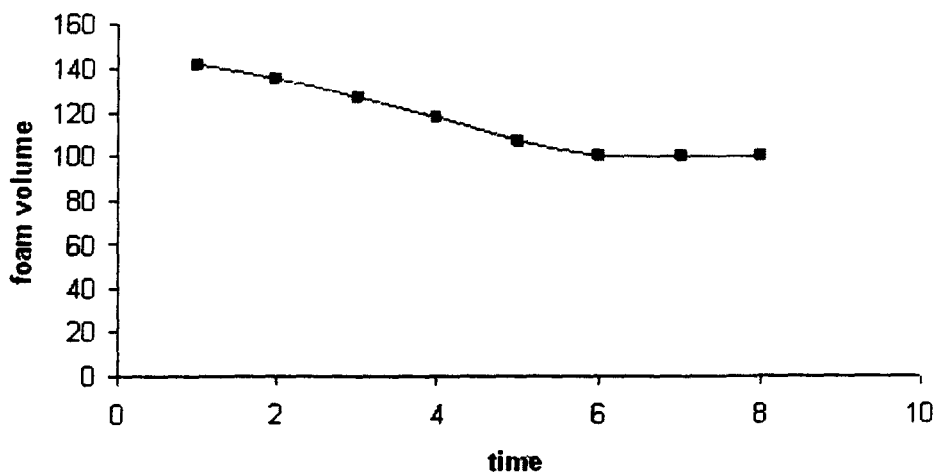




**Fig (1): Solubility of 0.5% (w/v) flaxseed mucilage at 20-95°C**

### Foam capacity

The foam capacity and stability of mucilage of aqueous dispersions of flaxseed is shown in Figure (2). Data revealed that the maximum time could be reached in order to get the maximum foam stability was 3.0 hours.



- Volume before whipping was 100 ml.
- Specific volume was 1,40 .
- Volume increase was 42%.

**Fig (2): Foam capacity of flaxseed mucilage**

The maximum foam capacity, however, was 142 ml, meaning 42% increase in the volume. For 1% (w/v) solution used in the present study, flaxseed mucilage gave foam values about 75% of those of ovalbumin and had similar time-dependent stability. The foam values of 0.5% and 0.1% solutions were 63 and 57% of the respective ovalbumin dispersions and they were far less stable over time as reported by Mazza and Biliaderis (1989).

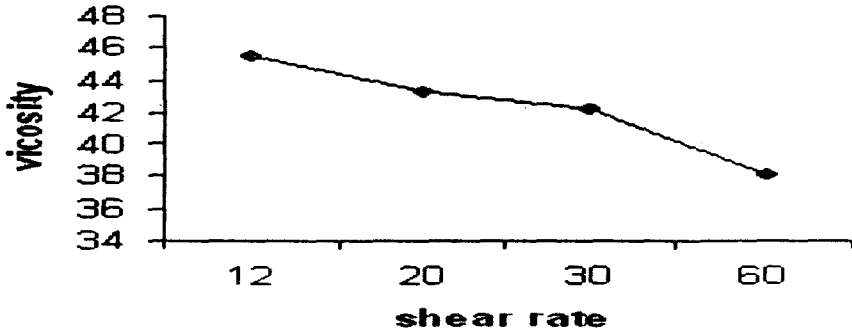
### **Viscosity**

The effect of shear rate on the apparent viscosity of mucilage at concentrations between 0.3 and 0.7 is shown in Fig. 3. Data revealed that viscosity of flaxseed mucilage decreased as the shear rate increased with all concentrations used. At low concentrations the flow curves tended to be a Newtonian behavior while at concentrations above 0.3% the solutions exhibited shear thinning with increasing shear rate, which is typical of polymeric solutions (Fig 3 a, b and c). Data also revealed that the concentration had pronounced effect on the apparent viscosity of mucilage. At the same shear rate, the apparent viscosity increased as the concentration of mucilage solution increased (i.e. the apparent viscosity was 45.5, 101.5, and 590 cp at concentration of 0.3, 0.5, and 0.7, respectively). When compared with other carbohydrate hydrocolloids at equal solids level (0.3% w/v), the flaxseed mucilage at low shear rates had intermediate viscosity between gum arabic and locust bean or guar gums (Mazza and Biliaderis, 1989). According to Bhatti (1993) the viscosity of mucilage extracts increased as the weight of flaxseed hulls in the dispersion is increased, thus providing a measure of mucilage content in the extract.

### **Scanning electron microscopy**

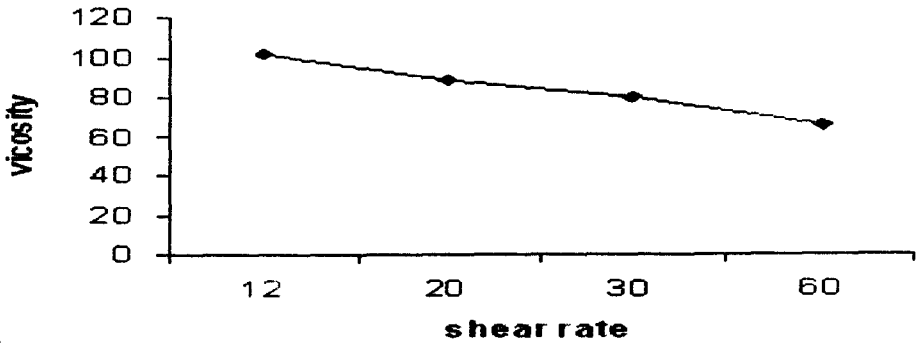
Flaxseed mucilage, a mixture of neutral and acidic polysaccharide, is present entirely in the seed coat, invariably called true hull or serpmoderm, the mucilage layers being on the outermost (epiderm) of the seed coat. The presence of mucilage in the outermost cells of the seed coat was readily apparent from scanning electron micrographs (Fig.4a, b) (Bahatty, 1993). The top part of the figure shows the outside of unsoaked seed coat fragment containing the mucilage, the cells were filled with the mucilage and the cell cellular structure was completely masked. The bottom part of the figure shows scanning electron micrographs of the seed coat after soaking in water. The mucilage dissolved in water, leaving behind empty-looking cells with clearly defined cell walls, the two micrographs provided a contrasting view of the outer layer of the seed coat. The soaked seed coat cells appeared pentagonal, many with unequal sides. According to Freeman (1992), the outermost layer of flaxseed coat varies in shape from rectangular to polygonal and is characteristic of the variety. Alix *et al.*

**concentration 0.3%**



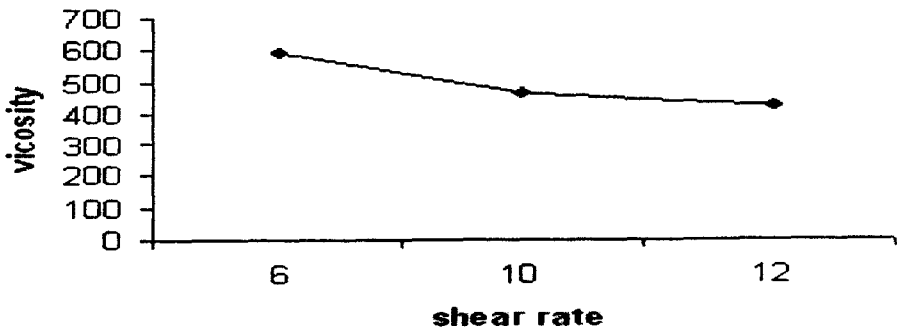
**A**

**concentraion 0.5%**



**b**

**cocentration 0.7%**



**C**

**Figure (3): Effect of shear rate on flaxseed mucilage Viscosity at different concentration**

(2008) have also observed this contrasting structural difference of flaxseed coat cells after soaking of hull fragments in water. In the present study, however, it was the first time to elucidate the microstructure of the mucilage using scanning electron microscope. The morphology of the section of the mucilage extracted at 100 to 25°C was examined by SEM (Fig. 5a,b). The extraction temperature did not influence the appearance of the mucilage. The mucilage appeared homogeneous and dense (Fig. 5a, b). No aggregates or granular appearance were detected in the samples indicating the complete homogeneity of the mucilage. No alteration of the mucilage appeared to be due to the cross linking conditions. The mucilage fibers were completely integrated into the mucilage matrix (Fig. 5a, b).

The solubility, foam stability and viscosity data suggested that flaxseed mucilage could be used as a substitute for gum arabic in food formulations. Flaxseed mucilage is commonly employed in cosmetic industry as texturing agent, however, in food industry, their application has not yet extensively examined. Polysaccharides extracted from flaxseed have shown promise as a novel food ingredient, however, very little is understood on its effect when added to food emulsions.

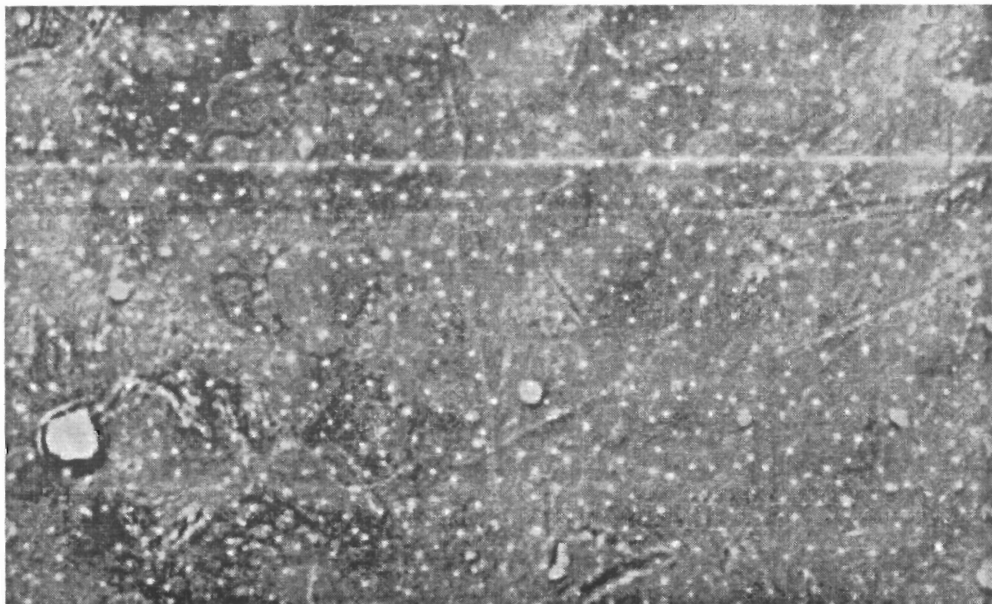
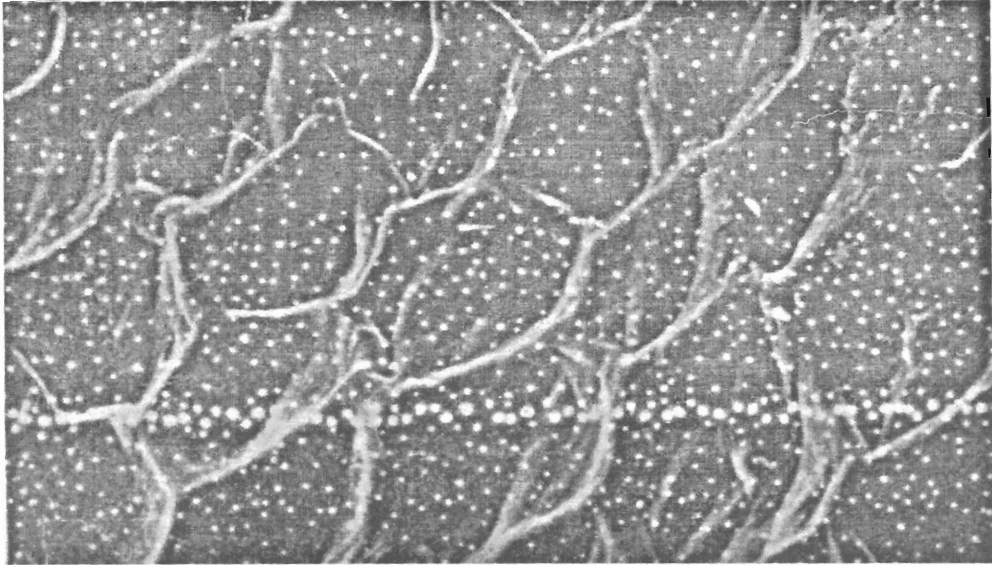


Fig.4a,b: Scanning electron microscopy of unsoaked and water-soaked fragments of seed coat of flaxseed, showing presence (top) and absence of mucilage (bottom). Magnification 328X, bottom, magnification 630X (from: Bahatty, 1993)

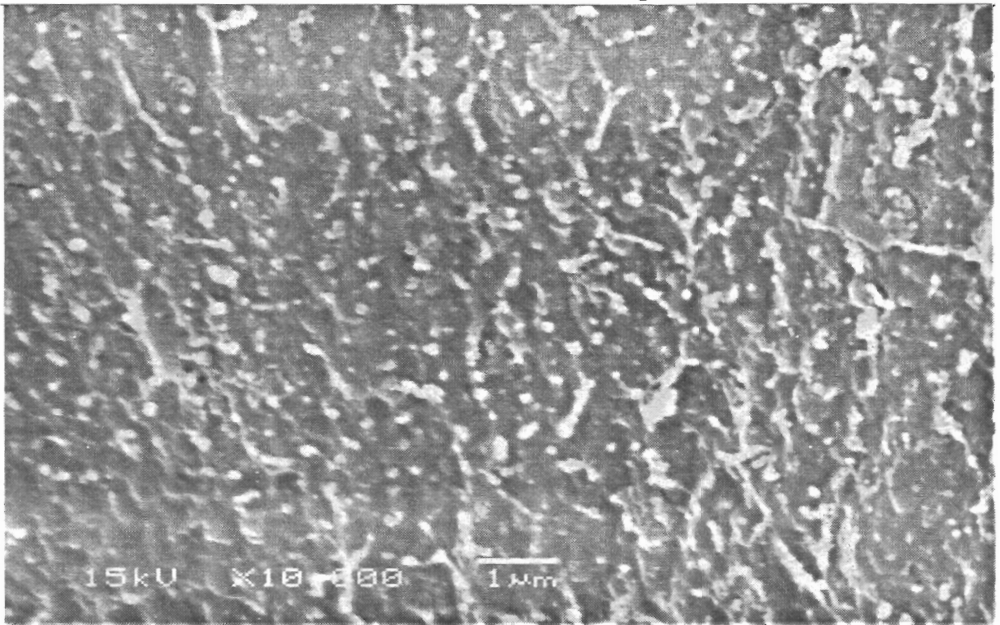


Fig. (5) a

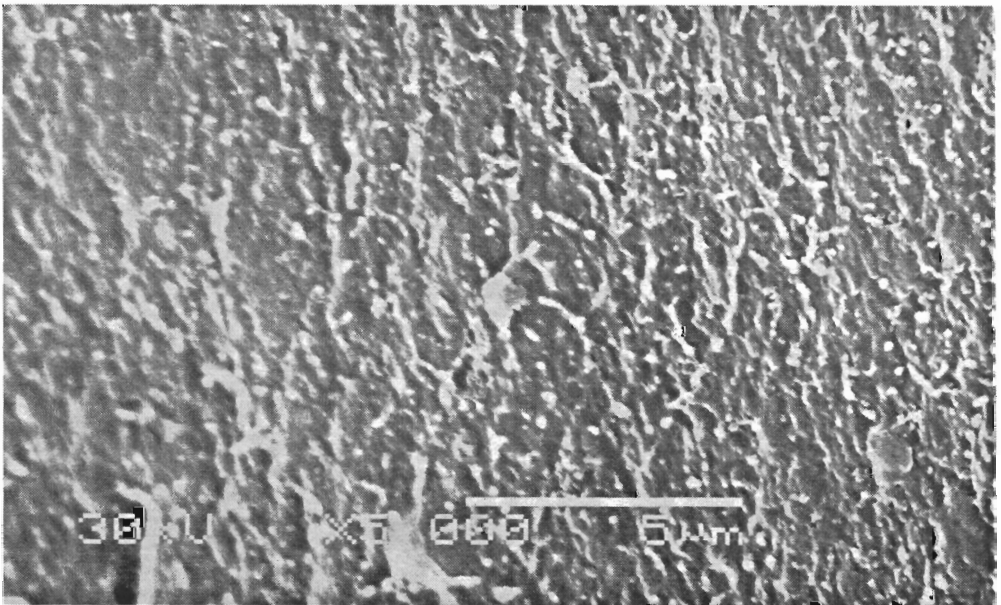


Fig. (5) b a microstructure of the mucilage using scanning electron microscope. The morphology of the section of the mucilage extracted at 100 to 25°C was examined by SEM .

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## الملخص العربي

### استخلاص موسيلاج بذرة الكتان وتركيبه و خواصه الطبيعية الكيميائية

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تم استخلاص موسيلاج بذرة الكتان من صنف بلينكا (*Linum usitaissium* L.) المنزرع في مصر باستخدام ماء بنسبة ١:٢٠ (W/V) علي درجة حرارة ٢٥ - ١٠٠ م. وتم ترسيب الموسيلاج بايثانول ٨٠%. وتم دراسة كل من التركيب الكيماوي وتركيب السكر و الخواص الوظيفيه لكل من الذوبان وسعة وثبات الرغوه واللزوجة والتركيب الدقيق. واطهرت النتائج ان الاستخلاص بالماء عند ٢٥ م انتج فقط من ٣-٥,٢ % موسيلاج بعد ٨ ساعات استخلاص بينما الاستخلاص بماء يغلي انتج ٨% في نفس الوقت. و كان المنتج النهائي في حاله الاخيره يعتبر لونه اغمق من الذي استخلص علي درجه حرارة الغرفة. ولذلك لخفض التلون البني وزيادة الناتج من الموسيلاج فان الاستخلاص تم علي درجة حرارة الغرفة ولكن البداية بماء مغلي. وبهذه الطريقه امكن استخلاص اكثر من ٩٠% من الموسيلاج خلال ٤ ساعات. وقد اتضح ان الموسيلاج الذي استخلص بماء يغلي عند ١٠٠ م اعطي اعلي في المحتوي من الزيت الخام و البروتين والرماد من الموسيلاج المستخلص علي درجة حرارة الغرفة. وقد بينت النتائج ايضا ان درجة حرارة الاستخلاص كان لها تاثير علي تركيب السكريات المعقدة. واطهرت النتائج ان ذائبية ٠,٥% في المحلول من الموسيلاج كانت بين ٧٠-٩٠% و اوضحت انها تكون ذائبه عند تركيز يصل الي ٠,٥%. وقد اعطي موسيلاج بذرة الكتان رغو حوالى ٧٥% من تلك المنتجه بالبومين البيض ولها نفس زمن الثبات. وقد انخفضت لزوجة موسيلاج بذرة الكتان كلما

زاد ال **shear rate** مع كل التركيزات المستخدمة . وعند التركيزات المنخفضة كانت منحنيات السريان تاخذ السلوك النيوتنيان **Newtonian behavior** بينما التركيزات الاعلي من ٠,٣% فان المحلول يحدث **shear thinning** مع زيادة ال **shear rate** والذي يطابق محاليل البوليمرات . وتبدل النتائج الخاصة بكل من الذاتييه وثبات الرغوه واللزوجه والتركيب الدقيق علي امكانية استخدام موسيلاج بذرة الكتان كبديل للصبغ العربي في الانظمة الغذائية .