

OPTIMIZATION OF ISOLATION FLAXSEED MUCILAGE FROM METHANOLIC EXTRACT AND ITS FUNCTIONAL CHARACTERISTICS

Kishk, Y. F. M.

Food Sci. Dept., Fac. Agric., Ain Shams Univ., Shoubra El-Kheima, Cairo, Egypt.

ABSTRACT

Composite design of three factors, isopropanol concentration, holding time and temperature were studied as an independent variables factors influencing in the isolation of flaxseed mucilage from methanolic extract. Rheological properties, emulsifying activity index (EAI), emulsion stability index (ESI), foam capacity and stability of isolated flaxseed mucilage (IFM) and crude flaxseed mucilage (CFM) were determined in comparison to other gums (Arabic gum, guar gum, carboxy methyl cellulose, xanthan or corn starch). Predicting individual isolation yield (Y) was assumed by quadratic polynomial regression model for the independent variables. A 60.6 % isopropanol concentration, 28.7 min. and 31.2 °C were the optimum conditions to produce the higher isolated flaxseed mucilage yield. Isolation process improved the dynamic viscosity of IFM compared to the CFM. Also IFM could be competing each of xanthan and guar gums. Flow behavior indexes (n) of the different polysaccharides were less than 1.0 indicating their pseudoplastic nature. IFM had higher consistency index (K) and lower n values than CFM implying that solution was more viscoelastic and shear thinning. IFM enhanced the EAI and ESI in a model system and came in the second order behind a guar gum while CFM came in the seventh order. IFM appeared a good ability to stabilize the foam model system whereas came in the first order compared to other polysaccharides. Color parameters were improved in the IFM compared to CFM with a significant differences ($P < 0.05$). It could be concluded that the IFM can be possible to use as a stabilizer or a thickening agent in food products.

Keywords: Flaxseed, Mucilage, Optimization, Gum, Extraction, Rheological properties, Emulsifying activity, Emulsion stability, Foam capacity, Foam stability.

INTRODUCTION

Flaxseed (*Linum usitatissimum*) is an annual plant of the linaceae family. The fruit contains a seed known as flaxseed or linseed (Pradhan *et al.* 2010). Flaxseed is one of the oldest crops known to man, and is cultivated for fiber and oil. It was used for medicinal purposes in ancient Egypt and Greece, mainly to relieve abdominal pains and also an energy source (Berglund, 2002; Kishk, 2004). The Egypt annual production of flaxseed during 2012 was 4000 tons (FAO, 2013).

Flaxseed is considered a source of functional ingredients, because it contains α -linolenic acid (Bozan and Temelli, 2008), lignans and polysaccharides (other than starch), all of which have positive effects in disease prevention (Bierenbaum *et al.*, 1993; Cacace and Mazza, 2006; Udenigwe, *et al.*, 2009). Flaxseed is one of the richest sources of plant lignans, being very rich in the lignan secoisolariciresinol diglucoside (SDG)

(Kordali *et al.*, 2005). It also contains small amounts of the lignans matairesinol, pinoresinol and isolariciresinol (Clevel and Bhatnagar 1992; Chen *et al.*, 1998). SDG, a lignan precursor, is converted by the bacterial flora of the human colon to two major mammalian lignans, enterodiol and enterolactone (Dietary phytoestrogens). Lignans are thought to exert protective effects by interfering with endogenous sex hormone metabolism (Adlercreutz *et al.*, 1981). Dietary phytoestrogens have been studied as potential compounds in cancer prevention (Oomaha, 2001; Prasad, 2005). Moreover, flaxseed mucilage works as an estrogen hormone in menopausal women (Ferguson *et al.*, 1989; Ayers and Loike, 1990). Enterolactone and enterodiol which are thought to have beneficial effects on human health, due to (anti) estrogenic, antiinflammatory, and antioxidative effects as well as the ability to reduce cancer and cardiovascular risks (Adlercreutz, 2007; Kitts *et al.*, 1999). Free radicals can damage tissues and have been implicated in the pathology of many diseases like atherosclerosis, cancer and Alzheimer's disease (Pratic, 2001). The antioxidant action of Seco and enterodiol is greater than that of vitamin E (Bhathena and Velasquez, 2002; Barbary *et al.*, 2010). Antioxidants are considered important nutraceuticals on account of many health benefits (Lee *et al.*, 2004; Touré and Xueming 2010).

Flaxseed mucilage appears a function characteristics resemble the Arabic gum (Fedeniuk and Biliaderis, 1994). Flaxseed mucilage is a heterogenic polysaccharide (Wanasundara and Shahidi, 1997). Dev and Quensel, (1988) reported that flaxseed can be employed in food systems to improve water binding and emulsifying properties. Food hydrocolloids are high molecular weight hydrophilic biopolymers used as functional ingredients in the food industry for the control of microstructure, texture, flavor and shelf-life (Dickinson, 2003). Flaxseeds are ranging in color from a deep brown to a light yellow (Daun, *et al.*, 2003). Seed color is determined by the amount of pigment in the outer seed coat – the more pigment, the darker the seed (Morris, 2007). Flaxseed mucilage is a carbohydrate gum like material associated with the hull of flaxseed (Bhatty and Cherdkiatgumchai, 1990). Flaxseed mucilage that extracted by 10 % ethanol had low brightness value (48.99) and high redness and yellowness values (9.34 and 22.66, respectively) (Kishk *et al.*, 2011). More darkness in the color of the cookies or pasta was observed as the level of the supplementation of the flaxseed flour (Hussain *et al.*, 2006; Gupta and Shivhare 2012). Previous studies differed in seed/ water ratio that used for extraction mucilage ranged between 1/10 (Wanasundara and Shahidi, 1997) to 1/40 (Singer *et al.*, 2011). Many researchers worked on the optimization of ethanol-water extraction of mucilage from flaxseed (Kishk 2004; Zhang *et al.*, 2007). In all previous studies the mucilage extract was dried without isolation from the extraction solution. As a result of this practice the extracted pigments, proteins and ash associated with the final mucilage product. It leads to produce dark mucilage and weakening functional properties. Stabilization of emulsions depending on the purity of the mucilage (Kadivar, 2001).

Therefore, the objectives of this study were to optimize the isolation process of flaxseed mucilage from extraction solution. Isopropanol concentrations, temperatures and holding times were used as independent

variables to define a range of variables necessary using the three dimension response surface method. The emulsifying, foaming and rheological properties of high yield resulting mucilage were evaluated in comparison to crud flaxseed mucilage, Arabic gum, guar gum, carboxy methyl cellulose, xanthan and corn starch were also evaluated.

MATERIALS AND METHODS

Materials

Brown flaxseed (*Linum usitatissimum*) obtained at harvest season 2012 from Agriculture Research Center, Ministry of Agriculture, Giza, Egypt. Also defatted soy bean flour purchased from Agriculture Research Center, Ministry of Agriculture, Giza, Egypt. Samples of xanthan gum (practical grade) and guar gum were obtained from Sigma chemical and Gumix International Companies. Carboxymethylcellulose, Arabic gum and corn starch were purchased from El-Gomhoria Company for Drugs and Chemicals, Cairo, Egypt. Albumin egg powder was obtained from El-Nasr Pharmaceutical Chemicals Company, Cairo, Egypt.

Methods

Mucilage extraction and isolation

Flaxseed mucilage was extracted at optimum conditions described by Kishk, (2004). Flaxseed was mixed with 10 % ethanol solution with a ratio 1/12. The temperature was raised to 67 °C with agitation on magnetic stirrer. The produced extract was dried in oven at 50 °C until constant weight. Then, grinded to powder particle size form and bottled in brown glass till used as crud flaxseed mucilage (CFM).

The different independent variables included isopropyl alcohol concentration, holding time and temperature were studied to produce the highest yield (dependent variable) from isolated flaxseed mucilage. Isopropyl alcohol was added to the flaxseed mucilage extract using different concentrations 0, 33, 50, 60 and 67 % (v/v) from the all final solution. In the same time effect of holding at different times (10, 20 and 30 min.) and different temperatures (30, 40 and 50 °C) were studied. The coagulated flaxseed mucilage dried at 50 °C then, grinded to the powder particle size form and bottled in brown glass tell used as isolated flaxseed mucilage (IFM).

Preparation of soy protein isolate

Soy protein isolate (SPI) was prepared according to the method described by Hirotsuka *et al.*, (1984). Twelve parts of hot water (50 °C) were added to one part of the defatted soy flour, and the pH was adjusted to 7.5 with 5 N NaOH. The mixture was stirred for one hour at 50 °C, then centrifuged at 3000 xg for 10 min. The supernatant was adjusted to pH 4.5 with 1 N HCl and centrifuged at 3000 xg for 10 min. The resulting precipitate was washed with a ten-fold volume of water and centrifuged at 3000 xg for 10 min. Finally, the precipitate was dissolved in an appropriate amount of water,

centrifuged and dried under vacuum at 50 °C for 12 hr. Thereafter, the SPI was ground and stored in dark bottle at room temperature till used.

Chemical analysis

Moisture, ash, crud lipid and protein (N x 5.70) analyses carried out according to the AACC (2000).

Rheological measurement

Viscosity and flow properties of CFM, IFM, Arabic gum, guar gum, carboxy methyl cellulose, xanthan and corn starch at concentration of 0.06 % were measured as described by Gladwell *et al.* (1985). The rotational viscometer rheo-test, type RV was used in a determination. The tested material was introduced into the "S1" cylinder of the viscometer at pH 7 and 25 °C. In such a case, shear stress was developed at shear ranging from 3.0 to 1312/sec. The dynamic viscosity was calculated according to the following formula:

$$\eta = \sigma / \gamma \quad (1)$$

Where: η = Dynamic viscosity in cp σ = Shear stress dyne/cm².

γ = Shear rate sec⁻¹

(σ) was calculated from the obtained torque value (α) and the cylinder constant Z (dyne/cm²) according to the following equation:

$$\sigma = Z \times \alpha \quad (2)$$

The power law parameters consistency index (K) and flow behavior index (n) were calculated from the regression coefficients of log shear rate and log shear stress data as mentioned by Paredes *et al.* (1988). The power law equation is as follows:

$$\sigma = K \cdot \gamma^n \quad (3)$$

Apparent viscosity (μ) at shear rate of 48.6 s⁻¹ was calculated from the experimental values as follows.

$$\mu = k \cdot \gamma^{n-1} \quad (4)$$

Emulsifying activity index and emulsion stability

Emulsifying activity index (m² g⁻¹) was determined at pH 7 using soy protein isolate (5 mg/ml water) as model system according to method introduced by Cameron *et al.*, (1991). Ten ml corn oil and 30 ml soy protein isolate aqueous solutions contained 0.05 % (w/v) of CFM, IFM, Arabic gum, guar gum, carboxy methyl cellulose, xanthan and corn starch were used. The mixtures were homogenized by warring blender for 60 sec., and then the emulsion transferred to the 100 ml glass beaker. 0.1 ml emulsion was immediately taken from the bottom of the beaker and diluted to 50 ml with 0.1 % sodium dodecyl sulfate. The absorbance of the diluted emulsion was measured at 500_{nm}. The initial A_{500nm} measurement was taken to be the emulsifying activity, while emulsion stability was measured at A_{500nm} after 20,

40 and 60 min. emulsifying activity index (EAI) and emulsion stability index (ESI) were calculated according the following equation:

$$\text{EAI or ESI} = \frac{2 \times 2.303 \times A_{500\text{nm}}}{25 \times L \times C} \quad (5)$$

Where: L, length of cuvette and C, concentration of polysaccharide

Foam capacity and stability

Foam capacity (ml) of CFM, IFM, Arabic gum, guar gum, carboxy methyl cellulose, xanthan and corn starch in egg albumin model system was determined according to method reported by Mott *et al.* (1999). The model system prepared by 0.05 % polysaccharide in 1 % egg albumin aqueous solution at pH 7. The volume of foam (ml) was measured at 30 sec. after whipping for 1 min. and was reported as foam capacity. The volume of foam after incubation at room temperature for 20, 40 and 60 min. was expressed as foam stability.

Color measurements

Color measurements (L^* , a^* , b^*) of CFM, IFM, Arabic gum, guar gum, carboxy methyl cellulose, xanthan and corn starch solutions (0.6 %) were analyzed with a colorimeter (Spectrophotometer MOM, 100 D, Budapest, Hungary) according to Humphries *et al.*, (2004). L^* values represent brightness on a 0 (pure black) and then 100 (pure white) scale, a^* values range from -60 (pure green) to +60 (pure red) and b^* values range from -60 (pure blue) to +60 (pure yellow). Each L^* , a^* and b^* value printed is averaged from three readings.

Statistical analysis

Optimum mucilage yield (Y) and the obtained data were analysed by nonlinear regression model. The model proposed for response of Y presented as follows:

$$Y = y_0 + aC + bT + ct + dC^2 \quad (6)$$

where y_0 , a, b, c and d are intercept and linear, quadratic regression coefficient terms, respectively. C (isopropanol concentration), T (temperature) and t (holding time) are independent variables. Analysis of variance was used to compare between means by Duncan multiple range at significance 5%. Means with different letters are significantly different. Anova and regression analysis (using PROC ANOVA and REG procedures) were carried out by Statistical Analysis System (SAS Program, 1996). Harvard Chart XI software (Harvard Chart XI Programme, 1999) was used to apply the three dimension response surface method to locate the optimum isopropanol concentration, temperature and holding time.

RESULTS AND DISCUSSION

Proximate composition

The chemical composition of CFM and IFM is presented in Table (1). Each of CFM and IFM characterized by low moisture content (5.6 and 6.1, respectively) with a significant difference ($P < 0.05$). The CFM had a high content of ash, protein and crud lipid. On the other hand, the ash, protein and crude lipid content were decreased with isolation process to the minimum percent in IFM. The ash and protein percent significantly ($P < 0.05$) decreased from 9.1 and 1.3 % in CFM to 2.3 and 0.7 % in IFM. The crud lipid not detected in an IFM. The nitrogen free extract content in IFM was significantly ($P < 0.05$) higher than that in a CFM with a values 90.9 and 83.0 %, respectively. According to the obtained data the isolation process lead to produce flaxseed mucilage with a high purity compared to the flaxseed mucilage that prepared by the traditional methods.

Table (1): Proximate chemical composition (%) of crude and isolated flaxseed mucilage.

Mucilage type	Moisture	Ash	Proteins N x 5.70	Crud lipid	NFE
CFM	5.8 ^a ± 0.21	9.1 ^a ± 0.35	1.3 ^a ± 0.20	0.8 ^a ± 0.06	83.0 ^b ± 0.49
IFM	6.1 ^a ± 0.56	2.3 ^b ± 0.21	0.7 ^b ± 0.08	0.0 ^b ± 0.00	90.9 ^a ± 0.63

- NFE; nitrogen free extract calculated by difference; CFM, crude flaxseed mucilage; IFM, isolated flaxseed mucilage
- Means in the same column with different letters are significantly different ($P < 0.05$).
- Values are mean ($n = 3$) ± standard deviations.

Optimization of mucilage isolation

The effects of isopropanol concentration (C), temperature (T) and holding time (t) on the isolated mucilage yield were studied by using three dimension response surface method which presented in Figure 1. Each isopropanol content in mucilage water extract and holding time had a significant effect on the mucilage yield (Fig. 1, A). Mucilage yield increased from 27.0 to 84.1 % with increasing the isopropanol concentration and time from 0.0 to 60.6 % (V/V) and 10 to 28.7 min. respectively at temperature 30°C. The polynomial predicted equation of different independent variables presents in (Eq. 1).

$$\text{Yield} = -129.0 + 6.1C + 0.32t - 0.046C^2 + 2.60t^2 \quad R^2 = 0.9882 \quad (7)$$

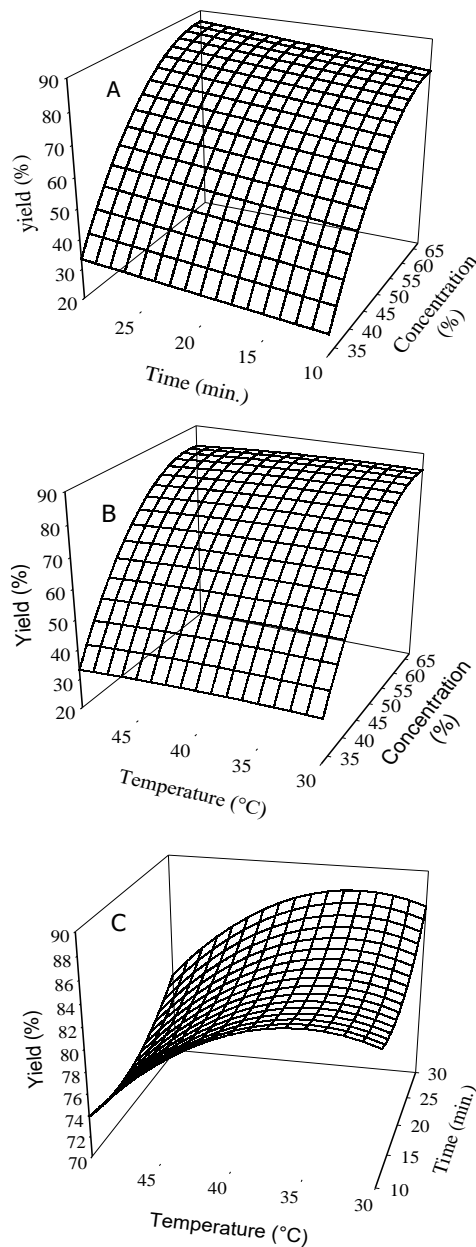


Figure (1): Predicted mucilage yield against different isopropanol concentrations and times at 30°C (A), isopropanol concentrations and temperatures 30 min (B) and times and temperatures at 67 % isopropanol concentration.

Effects of temperature and isopropanol concentration on the mucilage yield were studied at constant time 30 min. (Fig. 1, B). Mucilage yield was affected by each of the temperature and isopropanol concentration. The predicted values of different studied variables gave the highest mucilage yield (84.14 %) were 60.6 % isopropanol (V/V) and 31.2 °C. The predicted model (Eq. 2) reflects the relation between the variables.

$$\text{yield} = -132.1 + 6.4C + 0.55T - 0.049C^2 - 0.0078T^2 \quad R^2 = 0.9899 \quad (8)$$

Polynomial regression of the mucilage yield at different temperatures and times presents in Fig. 1, C. Upon the basis of apparent changes in extraction yield affected by temperature and time at concentration 67 %, a higher mucilage yield 86.4 % was observed at 31.2 °C and 28.7 min. The model linked between temperature and time shows in Eq. 3.

$$\text{Yield} = 35.2 - 0.65t + 3.0 T + 0.022t^2 - 0.043T^2 \quad R^2 = 0.7142 \quad (9)$$

Multiple regression coefficients were presented in Table (2) to predict polynomial model (Eq. 4) for isolated mucilage yield. The model was tested for adequacy by analysis of variance. The regression model for data were highly significant ($P < 0.01$) with $R^2 = 0.9654$. The coefficients of variation (CV) were $< 6.71\%$. The predicted model for extracted mucilage yield (Y) was reported as follows:

$$\text{Yield} = -111.5 + 5.790C - 0.295T + 0.403t - 0.043C^2 \quad (10)$$

Table (2): Regression coefficients of predicted quadratic polynomial model for response of the yield of purified flaxseed mucilage.

Parameters	Coefficients	Standard error
Linear		
a	-111.5	14.1
β1	5.790	0.577
β2	-0.295	0.088
β3	0.403	0.088
Quadratic		
β4	-0.043	0.006
R2	0.9654	
P value	<0.0001	
C.V %	6.71875	
R² = correlation coefficient C.V.= coefficient of variation		

Consequently, the obtained predicted model is possible for identify the optimum conditions required to produce a high yield of isolated mucilage from mucilage extract. It could be concluded that, the extraction by ethanol 10 % give a high mucilage yield than water (Kishk, 2004). However, 60.6 % isopropanol concentration at 31.2 °C for 28.7 min were the optimum conditions to produce a higher mucilage yield.

Rheological parameters

In comparison with other polysaccharides, CFM at low shear rates had intermediate viscosity (Figure, 2). Higher viscosity values were noticed for CFM in comparison with those of corn starch and Arabic gum and lower values than those of CMC, xanthan, IFM and guar gum, respectively. It can be observed, the isolated flaxseed mucilage from the extracted solution had a higher viscosity values than CFM. The CFM had a viscosity between each of the Arabic gum and guar gum. Mazza and Biliaderis, (1989) reported that the flaxseed mucilage at low shear rates had intermediated viscosity between gum Arabic and guar gum. On the other hand, the IFM viscosity was improved compared to the CFM. At high shear rates, differences in viscosity among different examined polysaccharides were maximized. The order of increasing viscosity was as follows: CMC> xanthan> IFM> guar gum> CFM> corn starch> Arabic gum (Figure, 3). The difference between the viscosity of IFM, guar gum and xanthan was negligible while, it was greater between IFM and CMC. The isolation process of IFM lead to improve its dynamic viscosity compared to other polysaccharides.

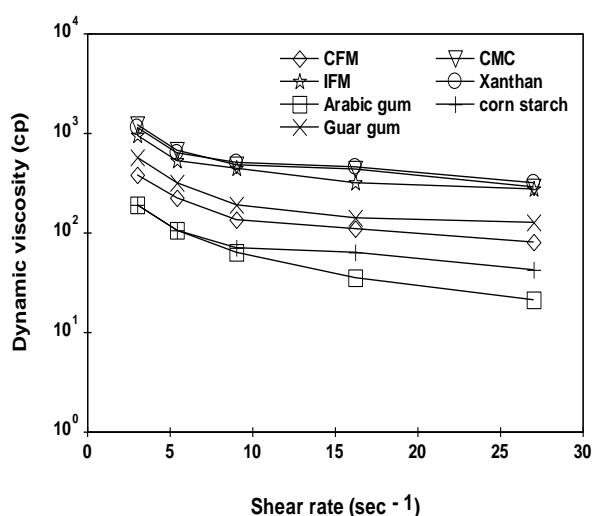


Figure (2): Flow curves of IFM, CFM and other commercial gums at low shear rate from 3 to 27 sec⁻¹.

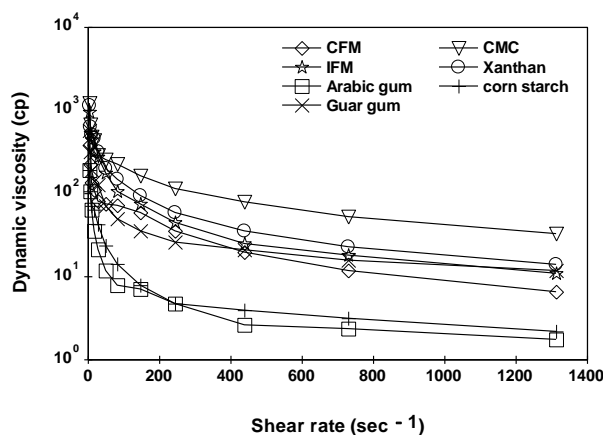


Figure (3): Flow curves of IFM, CFM and other commercial gums at shear rate from 3 to 1312 sec-1).

The flow behavior index (n) could be obtained from the slope of the double logarithmic plots of $\dot{\gamma}$ and σ , while consistency index (K) could be computed from the same plots by reading σ value at shear rate ranging from 3 to 1312 sec^{-1} . The results are given in Table (3) for CFM, IFM as well as other different studied gums. The determination coefficient (R^2) for the regression analyses of the log shear stress-log shear rate data was ranged between 0.8829 and 0.9863. It could be indicated that, the good fit was between the experimental data and model. The flow behavior indices (n) of the different tested solutions were less than 1.0 (ranging between 0.23 and 0.47) indicating their pseudoplastic nature.

Concerning the data presented in Table (3) the K value of the solution prepared using xanthan showed the highest value being 27.5. K values for solutions prepared using IFM and CMC were in the second and third order being 23.4 and 19.4, respectively. A K value of CFM was 18.6 which lower than the value of IFM.

According to the apparent viscosity, data recorded indicate that the IFM had a value 1.40 higher than Arabic gum, guar gum and corn starch that had values 0.16, 0.79 and 0.23, respectively. According to the previous presented data, the IFM can be compete the commercial polysaccharides like xanthan, Arabic gum, guar gum and corn starch in its food industry applications. This is in agreement with the fact that polysaccharide preparations of high intrinsic viscosity also exhibit greater shear thinning properties (Hales *et al.*, 1982 and Launay *et al.* 1986).

Table (3): Consistency index (*K*), flow behavior index (*n*) and apparent viscosity (μ) of crude, isolated flaxseed mucilage and other different polysaccharide.

polysaccharides	<i>K</i>	<i>n</i>	μ	R ²
CFM	18.6	0.33	1.36	0.9003
IFM	23.4	0.28	1.40	0.9585
Arabic gum	3.3	0.23	0.16	0.8829
Guar gum	8.9	0.38	0.79	0.9840
CMC	19.4	0.47	2.40	0.9863
Xanthan	27.5	0.29	1.80	0.9615
Corn starch	4.3	0.24	0.23	0.9266

CFM, crude flaxseed mucilage; IFM, isolated flaxseed mucilage; CMC, carboxy methyl cellulose.

Emulsifying activity and emulsion stability indices

Emulsifying activity and emulsion stability indices ($m^2 g^{-1}$) of CFM, IFM, Arabic gum, guar gum, carboxy methyl cellulose, xanthan and corn starch in model systems are presented in Table (4). The model system containing SPI (5 mg/ml aqueous solution) mixed with 0.05 % from the examined polysaccharide at pH 7.

All tested polysaccharides were characterized by significantly ($P < 0.05$) higher emulsion activity index than SPI ($55.1 m^2/g^{-1}$) in the model system. Emulsifying activity index of IFM significantly ($P < 0.05$) enhanced with value of $140.8 m^2/g^{-1}$ compared to CFM that had value of $76.7 m^2/g^{-1}$. In the same time the IFM could be competed the other commercial polysaccharides. The emulsifying activity index of polysaccharides ranged between $143.08 m^2/g^{-1}$ for guar gum to $105.58 m^2/g^{-1}$ for corn starch. The IFM was significantly ($P < 0.05$) being in the second order after guar gum that had the highest emulsifying activity index value. The emulsion stability indices in different model systems were gradually decreased during 60 min. of holding time with significant differences ($P < 0.05$). The emulsion stability index after 60 min. of emulsion that prepared using IFM was significantly ($P < 0.05$) better than the emulsion stability index of emulsion prepared using CFM with values were 87.6 and $31.1 m^2/g^{-1}$, respectively. In the same time, IFM appeared significantly ($P < 0.05$) high stability of emulsion during holding time with higher value than all of the studied polysaccharides except guar gum. Stabilize an emulsion depending on the purity of the mucilage (Kadivar, 2001). Guar gum emulsion came in the first order with high emulsion stability index being $97.9 m^2/g^{-1}$ after 60 min. In contrary, the CFM came in the fifth order compared to other polysaccharides with significant differences ($P < 0.05$). Associative interactions between proteins and polysaccharides lead to the formation of interpolymer complexes. Complex formation with charged polysaccharides, either anionic or cationic, gave soluble complexes substantially increase the emulsion stability of the both proteins (Tolstoguzov, 1998; Braudo *et al.*, 2001). Buffo *et al.* (2001) reported that, the most important colloidal interactions in emulsions are Van der Waals, electrostatic and polymeric steric.

Table (4): Emulsifying activity and emulsion stability indices ($\text{m}^2 \text{g}^{-1}$) of crude, isolated flaxseed mucilage and other different polysaccharides in model system (5 mg SPI/ ml aqueous solution + 0.05 % polysaccharide) at different times and pH 7.

Systems	EAI		ESI	
	zero	20 min.	40 min.	60 min.
SPI	55.1 ^h ± 0.3	53.6 ^g ± 0.4	36.6 ^f ± 0.1	44.7 ^e ± 0.1
CFM	76.7 ^g ± 0.3	38.5 ^h ± 0.1	47.1 ^g ± 0.6	31.1 ^f ± 1.1
IFM	140.8 ^b ± 0.6	123.1 ^b ± 0.1	109.4 ^b ± 0.4	87.6 ^b ± 0.3
Arabic gum	129.4 ^d ± 0.6	115.4 ^d ± 0.4	105.1 ^c ± 0.3	78.0 ^c ± 0.2
Guar gum	143.0 ^a ± 0.4	140.9 ^a ± 0.2	128.7 ^a ± 0.5	97.9 ^a ± 0.4
CMC	135.5 ^c ± 0.5	121.5 ^c ± 1.4	53.0 ^e ± 0.7	26.3 ^g ± 0.3
Xanthan	128.1 ^e ± 0.2	105.1 ^e ± 0.5	104.2 ^c ± 0.9	78.9 ^c ± 0.2
Corn starch	105.5 ^f ± 0.2	99.2 ^f ± 0.5	93.4 ^d ± 0.8	73.2 ^d ± 1.0

• EAI, emulsifying activity index; ESI, emulsion stability index; SPI, soya protein isolate; CFM, crude flaxseed mucilage; IFM, isolated flaxseed mucilage; CMC, carboxy methyl cellulose.

• Means in the same column with different letters are significantly different ($P < 0.05$).

• Values are mean ($n = 3$) ± standard deviations.

Foam capacity and stability

Foam capacity and stability (%) of CFM, IFM, Arabic gum, guar gum, carboxy methyl cellulose, xanthan and corn starch in model systems presented in Table 5. The model systems containing 1 % egg albumin mixed with 0.05 % CFM, IFM, Arabic gum, guar gum, carboxy methyl cellulose, xanthan and corn starch at pH 7. The model system contained IFM had significantly ($P < 0.05$) the highest foam capacity value 349.3 % compared to egg albumin and other polysaccharides. An aqueous mucilage solutions exhibits high solubility and good foam stabilizing properties (Mazza and Biliaderis, 1989). On contrary, CFM had the lowest foam capacity value (219.3 %). The foam capacity for other studied polysaccharides ranged between 224.0 to 333.0 %. On the other hand, with increasing the holding time, the foam volumes in all model systems were decreased (Table 5). The foam stability values were significantly reduced after 60 min. The foam stability (250.6 %) of solution prepared using IFM was significantly ($P < 0.05$) better than that of solutions prepared using CFM or other polysaccharides. In foams, the pressure in the dispersed phase is always higher than the pressure in the continuous phase. These pressure difference is given by the Laplace equation ($\Delta P = 2\gamma/r$) where γ is the interfacial tension and r is the radius of the gas bubbles. This capillary pressure causes drainage and thinning of the liquid film and eventual collapse of the film (Bergeron 1999; Stubenrauch and Klitzing 2003). According to the Laplace equation the IFM had positive effects on the interfacial tension and the gas bubbles radius to increase the thickening of the liquid film and prevents its thinning by decrease the pressure that lead to enhance the foam capacity and stability. On the other hand, the foam stability of 1 % egg albumin was significantly enhanced with the addition of flaxseed mucilage or other gums compared with foam stability of egg albumin solution. The main role on the stabilization of protein-polysaccharide stabilized interfaces was identified on the elasticity of the

interface (Miquelim *et al.*, 2010). IFM appeared good ability to stabilize the foam system was noticed for IFM whereas; CMC was in the second order with value 206.0 %. In this respect, Tolstoguzov (1998) indicated that protein-polysaccharides complex, as foam stabilizers are superior to protein alone because the complexes oppositely charged proteins. Generally, isolation of flaxseed mucilage from the extraction solution contributed in enhancement the role of IFM as foam stabilizer.

Mixtures of oppositely charged proteins and polysaccharides have a tremendous relevance in foods because they offer a unique possibility to adjust texture in gels and to stabilize surfaces and interfaces via electrostatic interactions, a process generally referred as coacervation (Onesippe and Lagerge, 2009). Furthermore, the electrostatic complexation of a protein with a polysaccharide can also provide a protective functionality, by preventing for example protein denaturation (Capitani, *et al.*, 2007). Generally, the performance of proteins alone in stabilizing foams can be improved by the addition of others stabilizing agents, usually polysaccharides (Narchi *et al.*, 2009).

Table (5): Foaming activity and foaming stability (Cm³) of crude, isolated flaxseed mucilage and other different polysaccharides in model system (10 mg egg albumin/ ml water + 0.05 % polysaccharide) at different times and pH 7.

Systems	Foaming activity		Foaming stability	
	zero	20 min.	40 min.	60 min.
Egg albumin	307.3 ^c ± 7.0	275.3 ^c ± 2.3	237.3 ^c ± 3.1	168.6 ^{cd} ± 8.1
CFM	219.3 ^e ± 4.2	162.6 ^g ± 6.4	146.6 ^g ± 7.0	104.0 ^e ± 6.9
IFM	349.3 ^a ± 7.0	290.0 ^b ± 9.2	271.3 ^a ± 2.3	250.6 ^a ± 6.4
Arabic gum	308.0 ^c ± 2.4	220.3 ^e ± 2.3	221.0 ^d ± 3.9	178.0 ^{cd} ± 10.9
Guar gum	312.0 ^c ± 0.0	245.6 ^d ± 2.5	222.6 ^d ± 2.1	184.6 ^c ± 11.3
CMC	333.0 ^b ± 4.8	321.6 ^a ± 0.3	257.0 ^b ± 5.0	206.0 ^b ± 14.8
Xanthan	248.6 ^d ± 5.0	186.6 ^f ± 8.1	172.0 ^f ± 5.3	166.6 ^d ± 6.1
Corn starch	224.0 ^e ± 4.0	197.0 ^f ± 8.9	209.0 ^e ± 6.3	171.6 ^{cd} ± 4.5

- CFM, crud flaxseed mucilage; IFM, isolated flaxseed mucilage; CMC, carboxy methyl cellulose.
- Means in the same column with different letters are significantly different ($P < 0.05$).
- Values are mean ($n = 3$) ± standard deviations.

Color measurements

Color parameters of CFM, IFM and other different polysaccharides are presented in Table (6). The L* (brightness) values of the IFM was significantly ($P < 0.05$) higher than the CFM. Whereas, the L* value enhanced from 22.4 in CFM to 25.0 in IFM. In the same time, the L* values of polysaccharides under this study ranged between 93.03 for corn starch to 15.30 for Arabic gum. It could be noticed that the L* value of IFM significantly ($P < 0.05$) rearranged to became in the third order while, the L* value for CFM became in the fifth order compared to other polysaccharides. On the other hand, the isolation process turned the b* (yellowness) value from the yellow direction to around the white

and black axis. This mean, the b^* value significantly ($P < 0.05$) improved from 3.06 in CFM to -0.93 in IFM. On contrary, the isolation process lead to decreases the value of a^* (redness) from -8.03 in CFM to -11.83 in IFM. The traditional method that used by Kishk ,(2004) depended on drying the mucilage extract by evaporation of water. This method leave pigments, minerals and protein with the dried flaxseed mucilage. Generally, using the optimum conditions to isolation flaxseed mucilage from the aqueous solution improved the color parameters of IFM compared to the CFM.

Table (6): Color parameter of crude, isolated flaxseed mucilage and other different polysaccharides.

Polysaccharides	a^*	b^*	L^*
CFM	-8.03 ^d ± 0.14	3.06 ^b ± 0.06	22.40 ^e ± 0.09
IFM	-11.83 ^f ± 0.40	-0.93 ^d ± 0.14	25.0 ^c ± 0.16
Arabic gum	-6.70 ^b ± 0.22	-0.56 ^c ± 0.05	15.30 ^f ± 0.61
Guar gum	-7.13 ^c ± 0.18	-2.93 ^f ± 0.6	23.50 ^d ± 0.23
CMC	-6.76 ^b ± 0.11	-2.03 ^e ± 0.16	15.80 ^f ± 0.24
Xanthan	-9.43 ^e ± 0.05	-6.10 ^g ± 0.02	31.56 ^b ± 0.05
Corn starch	0.51 ^a ± 0.4	5.10 ^a ± 0.20	93.03 ^a ± 0.64

- CFM, crud flaxseed mucilage; IFM, isolated flaxseed mucilage; CMC, carboxy methyl cellulose.
- Means in the same column with different letters are significantly different ($P < 0.05$).
- Values are mean ($n = 3$) ± standard deviations.

Conclusion

According to the response surface method, the best conditions to isolate of flaxseed mucilage was studied. The predicted variables were 60.6 % isopropanol concentration with holding time 28.7 min. at 31.2 °C. The isolation process was improved the IFM characteristics. The rheological, emulsifying, foam and color characteristics were significantly ($P < 0.05$) enhanced in IFM compared to the CFM and other examined polysaccharides. The obtained results recommended that the IFM it can be used in a wide range of different food types.

REFERENCES

- AACC. (2000). American Association of Cereal Chemists, Approved Methods of the AACC. St Paul: AACC. USA.
- Adlercreutz, H. (2007). Lignans and human health. *Critical Reviews in Clinical Lab. Sci.* 44, 483–525.
- Adlercreutz, H., Fotsis, T., Heikkinen, R., Dwyer, J.T., Goldin, B.R., Gorbach, S.L., Lawson, A.M., and Setchell, K.D. (1981). Diet and urinary excretion of lignans in female subjects. *Med. Biol.* 59, 259-261.
- Ayers, D.C., and Loike, J.U. (1990). Lignans: Chemical, Biological, and Clinical Properties, Cambridge University Press, Cambridge, UK, pp. 95–97.
- Barbary, O.M., El-Sohaimy, S.A., El-Saadani, M.A., and Zeitoun, A.M.A. (2010). Antioxidant, Antimicrobial and Anti-HCV Activities of Lignan Extracted from Flaxseed. *Res. J. Agric. Bio. Sci.*, 6, 247-256.

- Bergeron, V. (1999). Forces and structure in thin liquid soap films. *J. Phys. Condens Matter*. 11, R215–238.
- Berglund, D.R. (2002). Flax: New uses and demands. In Janick, J., Hipkey A., (Eds.), *Trends in new crops and new uses*. pp. 358–360.
- Bhathena, S.J., and Velasquez, M.T. (2002). Beneficial role of dietary phytoestrogens in obesity and diabetes. *Am. J. Clin. Nutr.* 76, 1191-1201.
- Bhatty, R. and Cherdkiatgumchai, P. (1990). Compositional analysis of laboratory-prepared and commercial samples of linseed meal and hull isolated from flax. *J. Amer. Oil Chem. Soc.* 67, 79-84.
- Bierenbaum, M.L., Reichstein, R., and Watkins, T.R. (1993). Reducing atherogenic risk in hyperlipemic humans with flax seed supplementation: A preliminary report. *J. Am. Coll. Nutr.* 12, 501-504.
- Bozan, B., and Temelli, F. (2008). Chemical composition and oxidative stability of flax, safflower and poppy seed and seed oils. *Bioresource Technol.* 99, 6354–6359.
- Braudo, E.E., Plashchina, I.G., and Schwenke, K.D. (2001). Plant protein interactions with polysaccharides and their influence on legume protein functionality. *Nahrung/Food*. 45, 382-384.
- Buffo, R.A., Reineccius, G.A. and Oehlert, G.W. (2001). Factors affecting the emulsifying and rheological properties of gum acacia in beverage emulsions. *Food Hydrocoll.*, 15, 53-66.
- Cacace, J.E., and Mazza, G. (2006). Pressurized low polarity water extraction of lignans from whole flaxseed. *J. Food Eng.* 77, 1087- 1095.
- Cameron, D.R., Weber, M.E., Idziak, E.S., Neufeld, R.J., and Cooper, D.G. (1991). Determination of Interfacial Areas in Emulsions Using Turbidimetric and Droplet Size Data: Correction of the Formula for Emulsifying Activity Index. *J. Agric. Food Chem.* 39, 655-659.
- Capitani, C., Perez, O.E., Pacheco, B., Teresa, M., and Pilosof, A.M.R. (2007). Influence of complex carboxy methyl cellulose on the thermo stability and gelation of α -lactalbumin and α -lactoglobulin. *Food Hydrocoll.*, 21, 1344–1354.
- Chen, H., Teng, L., Li, J., Park, R., Mold, D., Gnabre, J., Hwu, R., Tseng, W., and Huang, R. (1998). Antiviral activities of methylated nordihydroguaiaretic acid. 2. Targeting herpes simplex virus replication by the mutation insensitive transcription inhibitor tetra O-methyl-NDGA. *J. Med. Chem.* 41, 3001-3007.
- Cleveland, T.E., and Bhatnagar, D., (1992). Molecular strategies for reducing aflatoxins levels in crops before harvest. Bhatnagar, D., and Cleveland, T. (Eds.), *Molecular Approaches to Improving Food Quality and Safety*. Van Nostr and Reinhold, New York, USA, pp. 205-222.
- Daun, J.K., Barthet, V.J., Chornick, T.L., and Duguid, S. (2003). Structure, composition, and variety development of flaxseed. In: *Flaxseed in Human Nutrition*. Thompson, L.U., and Cunnane, S.C. (Eds.), 2th edition, AOCS Press, Champaign, IL, p 1-40.

- Dev, D.K. and Quensel, E. (1988). Preparation and functional properties of linseed protein products containing different levels of mucilage. *J. Food Sci.* 53, 1834-1837.
- Dickinson, E. (2003). Hydrocolloids at interfaces and the influence on the properties of dispersed systems. *Food Hydrocoll.* 17, 25-39.
- FAO. 2013. <http://faostat3.fao.org/home/>
- Fedeniuk, R.W., and Biliaderis, C.G. (1994). Composition and physicochemical properties of linseed (*Linum usitatissimum* L.) mucilage. *J. Agric. Food Chem.* 42, 240-247.
- Ferguson, K.J., Hoegh, C., and Johnson, S. (1989). Estrogen replacement therapy: a survey of women's knowledge and attitudes. *Arch. Intern. Med.* 149, 133–136.
- Gladwell, N., Grimson, M.J., Rahalkar, R.R., and Richmond, P. (1985). Rheological behavior of soy oil-water emulsions: dependence upon oil concentration. *J. Food Sci.* 50, 440-443.
- Gupta, R., and Shivhare, U. (2012). Optimization of process variables for the development of flaxseed and defatted flaxseed based pasta. *J. Nutr. Food Sci.* 2, 1-9.
- Hales, P.W., Jefferies, M., and Pass, G. (1982). Some physical properties of hydrocolloids in aqueous solution, In: *Gums and Stabilizers for the Food Industry*. Phillips, G.O., Wedlock, D.J., and Williams, P.A., (Eds.), Pergamon Press Inc., Maxwell House, Fairview Park, Elmsford, New York 10523, USA. pp, 33-43.
- Harvard Chart XI Progame. (1999). Version 3.0. USA: Lead Technologies Inc.
- Hirotsuka, M., Taniguchi, H., Narita, H., and Kito, M. (1984). Calcium fortification of soy milk with calcium-lecithin liposome system. *J. Food Sci.* 49, 1111-1112, 1127.
- Humphries, J.M., Graham, R.D. and Mares, D.J. (2004). Application of reflectance colour measurement to the estimation of carotene and lutein content in wheat and triticale. *J. Cereal Sci.* 40, 151–159.
- Hussain, S, Anjumf, M., Butt, M.S., Khan, M.I., and Asghar, A., (2006). Physical and sensoric attributes of flaxseed flour supplemented cookies. *Turk. J. Biol.*, 30, 87-92.
- Kadivar, M. (2001). Studies on integrated processes for the recovery of mucilage, hull, oil and protein from solin (low linolemc acid flax). PhD Thesis, Applied Microbiology and Food Science Department. University of Saskatchewan Saskatoon, Saskatchewan Canada.
- Kishk, Y.F.M. (2004). Optimization of flaxseed mucilage extraction and its functional characteristics. *Ann. Agric. Sci., Moshtohor*, 42 , 1177-1197.
- Kishk Y.F.M., Elsheshetawy, H.E., and Mahmoud, E.A.M. (2011). Influence of isolated flaxseed mucilage as a non-starch polysaccharide on noodle quality. *Int. J. Food Sci. Technol.* 46, 661–668.
- Kitts, D.D., Yuan, Y.V., Wijewickreme, A.N., and Thompson, L.U. (1999). Antioxidant activity of the flaxseed lignan secoisolariciresinol diglycoside and its mammalian lignanmetabolites enterodiol and enterolactone. *Mol. Cell. Biochem.* 202, 91-100.

- Kordali, S., Cakir, A., Mavi, A., Kilic, H., and Yildirim, A. (2005). Screening of chemical composition and antifungal and antioxidant activities of the essential oils from three Turkish *Artemisia* species. *J. Agric. Food Chem.* 53, 1408-1416.
- Launay, B., Doublier, L., and Cuvelier, G. (1986). Flow properties of aqueous solutions and dispersions of polysaccharides, In: *Functional Properties of Food Macromolecules*. Mitchell, J.A., and Ledward, D.A., (Eds.), Elsevier Applied Science Publishers, New York, USA.
- Lee, J., Koo, N., and Min, D.B. (2004). Reactive oxygen species, aging, and antioxidative nutraceuticals. *Comp. Rev. Food Sci. Food Saf.*, 3, 21-33
- Mazza, G. and Biliaderis, C. G. (1989). Functional Properties of Flax Seed Mucilage. *J. Food Sci.* 54, 1302–1305.
- Miquelim, J.N., Lannes, S.C.S., and Mezzenga, R. (2010). pH Influence on the stability of foams with protein–polysaccharide complexes at their interfaces. *Food Hydrocoll.* 24, 398-405.
- Morris, D.H. 2007. *Flax Primer - A Health and Nutrition Primer*, fourth edition. Published by Flax Council, Canada.
- Mott, C.L., Hettiarachchy, N.S., and Qi, M. (1999). Effect of xanthan gum on enhancing the foam properties of whey protein isolate. *J. Am. Oil Chem. Soc.* 76, 1383-1386.
- Narchi, I., Vial, C.H., and Djelveh, G. (2009). Effect of protein–polysaccharide mixtures on the continuous manufacturing of foamed food products. *Food Hydrocoll.* 23, 188–201.
- Onesippe, C., and Lagerge, S. (2009). Study of the complex formation between sodium dodecyl sulphate and gelatin. *Colloids and Surfaces A: Physicochem. Eng. Aspects.* 337, 61–66.
- Oomaha, B.D. (2001). Flaxseed as a functional food source. *J. Sci. Food Agric.* 81, 889-894.
- Paredes, M.D., Rao, M.A., and Bourne, M.C. (1988). Rheological characterization of salad dressing. 1- Steady shear, thixotropy and effect of temperature. *J. Text. Stud.* 19, 258-274.
- Pradhan, R., Meda, V., Rout, P., Naik, S., and Dalai, A. (2010). Supercritical CO₂ extraction of fatty oil from flaxseed and comparison with screw press expression and solvent extraction processes. *J. Food Eng.* 98, 393-397.
- Prasad, K. (2005). Hypocholesterolemic and antiatherosclerotic effect of flax lignan complex isolated from flaxseed. *Atherosclerosis* 179, 269-275.
- Pratic, D. (2001). In vivo measurement of the redox state. *J. Lipids* 36, S45-S47.
- SAS Program (1996). *SAS / STAT User's guide Release 6.12 Edition*. Cary NC, SAS Inst. Inc. USA.
- Singer, F.A.W., Taha, F.S., Mohamed, S.S., Gibril, A., and El-Nawawy, M. (2011). Preparation of mucilage/protein products from flaxseed. *Amr. J. Food Technol.* 6, 260-278.
- Stubenrauch, C., and Klitzing, R. (2003). Disjoining pressure in thin liquid foam and emulsion films—new concepts and perspectives. *J. Phys. Condens Matter.* 15, R1197–1232.

- Tolstoguzov, V.B. (1998). Functional properties of protein-polysaccharide mixtures. In: Function Properties of Food Macromolecules. Hill, S.E., Ledward, D.A., and Mitchell, J.R., (Eds.), 2th edition, Aspen Publishers, Inc. Gaithersburg, Maryland, USA, p. 261.
- Touré, A., and Xueming, X. (2010). Flaxseed Lignans: Source, Biosynthesis, Metabolism, Antioxidant Activity, Bio-Active Components, and Health Benefits. *Comp. Rev. Food Sci. Food Saf.* 9, 261-269.
- Udenigwe, C., Lu, Y., Han, C., Hou, W., and Aluko, R. (2009). Flaxseed protein-derived peptide fractions: Antioxidant properties and inhibition of lipopolysaccharide-induced nitric oxide production in murine macrophages. *Food Chem.* 116, 277–284.
- Wanasundara, P.K.J.P.D., and Shahidi, F. (1997). Removal of flaxseed mucilage by chemical and enzymatic treatments. *Food chem.* 59, 47-55.
- Zhang, z., Li, D., Wang, L., Ozkan, N., Chen X.D., Mao, Z., and Yang H. (2007). Optimization of ethanol–water extraction of lignans from flaxseed. *Sep. Pur.Technol.* 57, 17–24.

الظروف المثلى لعزل صمغ بذور الكتان من المستخلص الكحولي وخصائصه الوظيفية

ياسر فكرى محمد كشك

قسم علوم الأغذية – كلية الزراعة – جامعة عين شمس – القاهرة – مصر

أجرى هذا البحث بهدف دراسة تأثير ثلاثة من المتغيرات المستقلة وهي تركيز كحول الايزوبروبانول، وقت الحجز و درجة الحرارة على كفاءة عزل صمغ بذور الكتان من مستخلصه الكحولى. وقد تم تقدير الخصائص الريولوجية، معامل النشاط الإستحلابى، معامل ثبات المستحلب، القدرة على تكوين الرغوة وثبات الرغوة لكل من صمغ بذور الكتان المعزول والخام وذلك بالمقارنة ببعض السكريات الأخرى المستخدمة على النطاق الصناعى مثل (الصمغ العربى، صمغ الجوار، كربوكسى ميثيل سليلوز، الزنثان ونشا الذرة). قد تم إستنباط نماذج رياضية للتنبؤ بكمية الصمغ المعزولة بإستخدام نموذج تحليل الإنحدار التريبعى المتعدد الحدود للمتغيرات المستقلة. وجد أن أفضل ظروف تعطى أعلى ناتج لصمغ بذور الكتان المعزول تركيز 60,6 % ايزوبروبانول، وزمن حجز 28,7 دقيقة ودرجة حرارة 31.2^oم. وأدت عملية عزل صمغ بذور الكتان الى تحسين اللزوجة الديناميكية مقارنة بالصمغ الخام كذلك فقد وجد أن لمعزول صمغ بذور الكتان القدرة على منافسة كل من الزنثان وصمغ الجوار. وأن معامل الإنسياب لكل السكريات العديدة تحت الدراسة أقل من الواحد الصحيح دلالة على الطبيعة الشبه بلاستيكية. تميز معزول صمغ بذور الكتان بارتفاع معامل القوام وإنخفاض معامل الإنسياب مقارنة بالصمغ الخام دلالة على قدرته على تكوين محلول ذو لزوجة أعلى منه. وقد حسن الصمغ المعزول كل من معامل النشاط الإستحلابى ومعامل ثبات المستحلب فى نموذج الإستحلاب المحضر وجاء فى المرتبة الثانية بعد صمغ الجوار بينما جاء الصمغ الخام فى المرتبة السابعة مقارنة بالسكريات العديدة الأخرى. أظهر معزول صمغ بذور الكتان قابلية جيدة فى زيادة ثبات الرغوة فى نموذج الرغوة المحضر، وقد جاء فى المرتبة الأولى مقارنة بالسكريات العديدة الأخرى. كما تحسن معنويا ($p < 0.05$) اللون فى الصمغ المعزول مقارنة بالصمغ الخام. ويمكن القول بأن معزول صمغ بذور الكتان يمكن إستخدامه كمثبت للمستحلب والرغوة وكمغلظ للقوام فى المنتجات الغذائية.

قام بتحكيم البحث

كلية الزراعة – جامعة المنصورة
كلية الزراعة – جامعة عين شمس

أ.د / احمد عبد العزيز الرفاعى
أ.د / نجوى موسى حسن