American Journal of Food Technology

ISSN 1557-4571

Academic Journals Inc.

www.academicjournals.com

American Journal of Food Technology 6 (4): 260-278, 2011
ISSN 1557-4571 / DOI: 10.3923/ajft.2011.260.278

Preparation of Mucilage/Protein Products from Flaxseed

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ABSTRACT

This study aimed at obtaining a functional food ingredient in the form of a high mucilage product from hexane defatted isopropanol-detoxified flaxseed meal, hexane defatted-methanol detoxified flaxseed meal and isopropanol defatted flaxseed meal. The mucilage product was prepared by four methods namely: (1) The coprecipitate method, which gave a mucilage/protein product M1, (2) The modified coprecipitate method that resulted in mucilage/protein product M2, (3) The boiling water method that gave rise to mucilage/protein product M3 and (4) The enzymatic treatment method which led to obtain a mucilage/protein product M4. All mucilage/protein products were free of cyanogenic glycosides. Results indicated that M2 contained the highest content of soluble dietary fiber (mucilage) and little protein. Optimum conditions for the production of M2 include: the use of the modified coprecipitate method at a meal: water ratio of 1:40, a mucilage :ethanol ratio of 1:50 and a temperature of 5°C. The antioxidant activities of the four mucilage protein products were in the following order M2>M1>M3>M4. M2 was chosen for further study. Functional properties of M2 revealed poor wetting ability, poor gelling property, reasonable flowability, oil holding capacity, good emulsifying property, foam stability, excellent water absorption capacity. M2 was then incorporated into tap water at different concentrations to give a functional food product fiber water which was stored for three months. Microbiological examination of the stored fiber water revealed no growth of microorganisms. Sensory evaluation of stored samples indicated that color was not affected, also no significant difference of odor and consistency mean values were detected. On the other hand, the taste and overall acceptability mean values were significantly affected.

Key words: Flaxseed, soluble fiber, antioxidant, functional food, fiber water

INTRODUCTION

Flaxseed is ranked among the very important functional foods because of its high content of α-linolenic acid, dietary fiber, lignans, flavonoids, phenolic acids and good quality protein (Berglund, 2002). Flaxseed and flaxseed meal were proved to possess appreciable amounts of total dietary fiber between 38.9-40.3% as reported by Hettiarachchy *et al.* (1990), Berglund (2002). Although, most of the dietary fiber in flaxseed is insoluble 27-36%, yet it is considered a rich source of soluble dietary fiber (mucilage) 7-14% (Hadley *et al.*, 1992). Yet much variation in the content of water soluble polysaccharides in flaxseed from different geographical regions and cultivars were reported (Oomah *et al.*, 1995; Diederichsen *et al.*, 2006). The hindrance to the use of flaxseed and flaxseed meal in nutrition is the presence of cyanogenic glycosides which release toxic HCN upon hydrolysis (Feng *et al.*, 2003). Several methods have been reported for the removal of cyanogenic glycosides (Yang *et al.*, 2004).

Flaxseed is unique among oilseeds and cereals due to its high content of mucilage or gum located in the outer layers of the seeds. Mucilage belongs to the soluble fraction of dietary fiber (DF). Mucilage appears to play a role in reducing diabetes and coronary heart disease, preventing colon and rectal cancer and reducing the incidence of obesity (Franklin, 2009). The soluble fiber of flax can make bowel elimination easier while increasing its volume and reduce irritation at the same time, even for people with irritable bowel or problems with diverticulas (Tarpila *et al*., 2005). Flaxseed contains 40% TDF, compared to 17, 11, 49, 78 and 75% Total Dietary Fiber (TDF) in oat bran, oat meal, wheat bran, corn bran and rice bran, respectively. Ten percent of the TDF of flaxseed are soluble dietary fiber (SDF), while 30% are insoluble dietary fiber (IDF). Oat bran follows with 8% SDF and 8% IDF. Other brans contain only 3-5% of their TDF in the soluble form (Oomah and Mazza, 1998). Analysis of flaxseed mucilage revealed 4.57% moisture, 95.43% dry weight, 8.6% yield, 5.8% ash and 12.3% protein (Khayami *et al*., 2008).

Polysaccharides (mucilage) are generally obtained by aqueous extraction of the whole seed or meal. Yield, rheological properties and compositional characteristics of the extracted gum are dependent upon the pH, seed: water ratio, temperature and processing time (Koochecki *et al*., 2009). Flaxseed mucilage was extracted from whole flaxseed with hot water (Mazza and Oomah, 1995; Thakur *et al*., 2009), followed by precipitation with ethanol and freeze-drying (Mazza and Oomah, 1995), or separated by filtration (Mitra, 2002) .The freeze dried, material is relatively pure, free of antinutrients and stable on storage. Somboonpanyakul *et al*. (2006) extracted gums from malva nut in a sequential manner using water, 0.05 M H Cl and 0.05 M NaOH solutions, then analysed and further characterized the alkaline fraction. Wanasundara and Shahidi (1997) removed mucilage from flaxseed by soaking in water or sodium bicarbonate solutions or treatment with commercially available carbohydrases. Dev and Quensel (1988) extracted flaxseed mucilage as well as flaxseed protein at alkaline pH (9-12) then precipitated both near the IEP of the protein, washed at neutral pH then dried the mucilage product. Kankaanpaa-antilla (1999) in their patent suggested the use of an acid and an alkanol to precipitate proteins and mucilage from the alkaline extract. Holmgren (1990) patented an enzymatic hydrolysis method for the preparation of a mucilage product with low protein content. He used phytase enzyme to hydrolyze the phytates, a protease to hydrolyse the protein and an amylase for starch removal and he ended up with a dietary fiber product which had high swelling ability and water retention. Yunosov and Steven (2009) also reported on the sequential use of different enzymes, namely, viscozyme, protease and amylase for the preparation of mucilage product from several seeds. Wanasundara and Shahidi (1997) and Wu *et al*. (2009), recommended the use of enzymes such as Pectinex Smash XXL, celluclast*1.5 L, Ultra SP and Viscozyme during the extraction of mucilage. Also, Li *et al*. (2007) recommended the use of ultrasound-assissted extraction of the polysaccharides from different tissues of plant material.

Polysaccharide gums and mucilages are of commercial importance in the food industry because of their good emulsifying properties, where they are used for stabilization of emulsions, suspension of particulates, control of crystalization, encapsulation, formation of films and thickening (Parawira, 2008). The food options for flaxseed incorporation are many-from vegetable soups and stews to breakfast cereals, breads and muffins (Jenkins, 1995). Several authors have investigated the incorporation of flaxseed into muffins, bread and bakery products (Shearer *et al*., 2005; Mente *et al*., 2008; Lipilina and Ganj, 2009). Employing flaxseed mucilage in selected food systems (i.e., fish sauce, ice cream and meat emulsions) contributed to improved water binding and emulsifying properties. Meat emulsions extended with flaxseed mucilage showed reduced cooking loss and reduced firmness (Dev and Quensel, 1988).

Because of the many health benefits of mucilage, the aim of the present work was to prepare high mucilage product from hexane defatted detoxified flaxseed meal and to incorporate the mucilage into drinking water as it is the best vehicle for reaching consumers who need it.

MATERIALS AND METHODS

Flaxseed meal: Flaxseed (*Linum usitatissimum* L.), type Giza 8, were obtained from the Department of Oil Crops, Ministry of Agriculture, Dokki, Egypt, (Crop of 2006). Ground flaxseed was cold pressed using a hydraulic press. Residual oil in the flaxseed cake resulting after pressing was removed by extraction with n-hexane to give hexane defatted flaxseed meal (HDFM) or with isopropanol to give isopropanol defatted flaxseed meal (IDFM), by the use of a soxhlet apparatus. HDFM was detoxified using isopropanol (HDFM-I). The resulting flaxseed meals were dried, ground and saved for further work.

Enzymes: Proteinase and amylase were products of Sigma Aldrich Chemical Co.

Proteinase from *Aspergillus oryzae*, activity 3.9 Units mg^{-1} Solid, pH 7.0, temperature 70°C. α-Amylase from *Bacillus subtilis*, activity 380 Units mg^{-1} solid, pH 6.0, temperature 68-70°C.

Analytical methods: Analytical analysis was carried out on flaxseed, defatted flaxseed meals, detoxified flaxseed meals and mucilage products.

Moisture, oil, protein, ash, crude fiber contents were determined according to AOAC (2005) Total Dietary Fiber (TDF) was determined according to the method described by AOAC (2005). Soluble dietary fiber (SDF) and insoluble dietary fiber (IDF) were determined according to the method described by AACC (1990). Evaluation of antioxidant activity based on coupled oxidation of β-carotene/linoleic acid system was conducted according to Al-Salkhan *et al.* (1995).

Functional properties of the mucilage: Wettability, flowability and bulk density were estimated according to Taha and Ibrahim (2002). Water Absorption Capacity (WAC) according to (Huber, 1982). Oil Holding Capacity (OHC) according to Childs and Forte (1976). Emulsifying Capacity (EC) as indicated by Shahidi *et al.* (1995). Foam Stability (FS) as described by AACC (1990). Gelation according to Circle *et al.* (1964).

Microbiological analysis: Total viable bacterial count, spore former bacterial count, coliform count and yeast and molds count were carried out according to APHA (1992),using nutrient agar medium for the first 2 tests ,VRB agar and potato dextrose agar for coliform,yeast and molds count, respectively (Oxoid Mannual, 1991). Incubation temperature was 30, 30, 37 and 28°C for viable bacterial count, spore former bacterial count, coliform count and yeast and mold count, respectively, while incubation time was 48 h in all tests.

All types of analysis were carried out in triplicates and experiments replicated twice.

Sensory evaluation: Fiber water samples prepared by the addition of different mucilage concentration were assessed for their quality attributes by ten members of trained preference taste panel. The samples were introduced to the taste panel every week of storage after 48 h of the microbiological analysis and getting its data, to avoid testing of microbiologically unsafe samples.

The panelists were asked to score consistency, taste, color, odor, overall acceptability and emulsion separation by giving a degree from ten according to Raganna (1977).

Statistical analysis: The data were analyzed according to user's Guide of Statistical Analysis System (SAS, 1996) at the computer center of Faculty of Agriculture, Ain Shams University.

Experimental methods: Four procedures were used for the extraction of mucilage as follows:

Preparation of mucilage by coprecipitate method: Mucilage and protein were extracted from the hexane defatted flaxseed meal (HDFM) according to Dev and Quensel (1988) with a few modifications as follows:

HDFM was dispersed in distilled water using a meal to water ratio of 1:40 (w:v).The pH of the slurry was adjusted to pH 9 with 0.5 N Sodium hydroxide solution , stirred for 30 min at room temperature then centrifuged at 2500xg for 30 min. The supernatant was filtered through a glass wool and its pH adjusted to 4.8 by the addition of 0.5 N Hydrochloric acid then centrifuged at 4500 xg for 30 min. The final precipitate was suspended in a small volume of distilled water, adjusted to pH 7 with 0.5 N sodium hydroxide, then freeze dried in a freeze dryer (Va C05, Zirbus Technology, Osterode, Germany). The freeze dried powder was stored in containers over activated silica gel in a desiccator. This mucilage/protein product is designated (M1).

Preparation of mucilage by modified coprecipitate method: HDFM was dispersed in distilled water using a defatted meal to water ratio of 1:40 (w:v). The pH of the slurry was adjusted to pH 9 with 0.5 N Sodium hydroxide solution, stirred for 30 min at room temperature then. The slurry was centrifuged under cooling at 2500 xg for 30 min and the supernatant was filtered and the mucilage was precipitated by addition of ethanol and allowing the ethanol extract to stand for 24 h at 5°C. The precipitated mucilage was separated by centrifugation under cooling at 4500 xg for 30 min at 5°C, then dissolved in water and adjusted to pH 7 by 0.5 N sodium hydroxide. The concentrate was freeze dried in a freeze dryer (Va C05, Zirbus Technology, Osterode, Germany). The dried powder was stored in closed containers over activated silica gel in a dessicator. The mucilage product is designated (M2).

Preparation of mucilage by boiling water method: Mucilage was extracted using the boiling water method from HDFM according to Bhatty (1993) with some modification as follows:

HDFM was extracted with the boiling water at 100°C for 30 min, using a flaxseed meal to water ratio of 1: 40 (w: v). Extraction was carried out by electric stirring of the mixture. The extract was left to cool to room temperature. The extract was then centrifuged at 4500 xg for 30 min at 5°C and filtered through glass wool. The mucilage was precipitated from the extract by adding ethanol. After allowing standing for 24 h at 5°C, the precipitate was removed by centrifugation at 4500x g for 30 min at 5°C, final precipitate was homogenized in little water and adjusted to pH 7 by 0.5 N sodium hydroxide. The concentrate was freeze dried in a freeze dryer (Va C05, Zirbus Technology, Osterode, Germany). The freeze dried mucilage powder was stored in closed containers over activated silica gel in a dessicator. The mucilage product is designated (M3).

Preparation of mucilage by enzymatic method: Mucilage was prepared according to Holmgren (1990) as follows:

Defatted flaxseed meal was dispersed in water at a ratio of 1:40 (w:v) . The slurry was adjusted to pH~6-7, with continuous stirring and the temperature was adjusted to 70°C. A proteinase enzyme was then added (0.1 g/100 g meal) the pH, temperature were maintained through out this

step until the protein content in the mixture was reduced to less than 8%. Aliquots were taken at intervals for protein determination. AT the end of the hydrolysis, the pH was decreased to 4 and the temperature raised to 85°C to inactive enzyme. In a following step the temperature was kept at 68-70°C and pH adjusted to ~6-8, then a starch degrading enzyme (α-amylase) was added at concentration of 0.1 g/100 g meal and pH, temperature kept constant until most of the starch has been degraded. Aliquots were taken at intervals and tested by potassium iodide solution for starch detection. The temperature was then raised to 90°C and pH decreased to 4.8 to inactivate enzyme then the mixture was freeze dried in a freeze dryer (Va C05, Zirbus Technology, Osterode, Germany). The mucilage product is designated (M4).

Preparation of fiber water samples: Tap water used for preparation of water samples was collected from running tap water after 15 min of running to avoid using of standing water in pipelines. Aliquots of water or water mixed with 0.01, 0.03 and 0.05% mucilage were filled in bottles that were thermally processed in boiling water for 20 min, sealed with screw caps and stored in refrigerator at 6±2°C.

RESULTS

The health and nutritional benefits of flaxseed mucilage made it worthwhile to prepare a flaxseed mucilage product. However the presence of polysaccharides (mucilage) in the seed coat may hinder the separation of proteins due to their swelling in the aqueous medium (Smith *et al*., 1946; Sosulski and Bakal, 1969). It was also concluded from a study that the removal of seed coat polysaccharides had a major effect on enhancing protein yield of flaxseed meal via solubilization process (Wanasundara and Shahidi, 1997). The complete separation of mucilage from protein is rather difficult and thus the preparation of a pure mucilage product would be rather impossible. Therefore instead of a mucilage product a mucilage/protein product will be prepared.

Compositional analysis of mucilage/protein products: Compositional analysis of the four mucilage/protein products M1, M2, M3, M4 prepared from (IDFM), are presented in Table 1. Mucilage/protein product M1 prepared by the coprecipitate method contained 62.9% protein, oil content negligible, ash 9.5% and no Cyanogenic Glycosides (CG) detected. Mucilage/protein product M2 prepared by the modified coprecipitate method from IDFM contained 8.5% protein, 10.2% ash, negligible oil and no CG detected. Mucilage/ Protein product M3 prepared by the water boiling method contained 20.1% protein, less ash 6.4%, no CG and negligible oil. As for Mucilage /protein product M4 prepared by the enzymatic method it contained 7.8% protein, 5.9% ash no oil no CG.

Table 2 represents compositional analysis of M1, M2, M3 and M4 prepared from HDFM-MM. Mucilage/protein products M1, M2, M3 and M4 contained 65, 8.7, 18.7 and 7.3% protein, 9.3, 10.6, 12.3 and 9.2% ash, respectively. Oil content in all samples is negligible and no CG in all samples detected.

Table 3 indicates values of protein, ash, oil content, CG content of M1, M2, M3 and M4 prepared from HDFM-I. Mucilage protein product M1 contains 64.9% protein and 9.3% ash, M2 contains 8.6% protein and 9.8% ash, M3 and M4 contain 19.8 and 7.8% protein,11, 2 and 9.4% ash, respectively, 0.5-0.3% residual oil and no CG. Mucilage/protein.

Table 1: Compositional analysis of mucilage/protein products prepared by four methods from isopropanol defatted flaxseed meal (IDFM)

Mucilage /protein product	Protein (%)	Oil (%)	Ash (%)	CG as HCN
M_1	62.9	0.5	9.5	ND
M_2	8.5	0.2	10.2	ND
M_3	20.1	0.3	6.4	ND
M_4	7.8	0.3	5.9	ND

M_1: Mucilage prepared from HDFM by coprecipitate method; M_2: Mucilage prepared from HDFM by modified coprecipitate method; M_3: Mucilage prepared from HDFM by boiling water method; M_4: Mucilage prepared from HDFM by enzymatic method

Table 2: Compositional analysis of mucilage/protein products prepared by four methods from hexane defatted meal detoxified using methanol mixture (HDFM-MM)

Mucilage /protein product	Protein (%)	Oil (%)	Ash (%)	CG as HCN
M_1	65.0	0.5	9.3	ND
M_2	8.7	0.3	10.6	ND
M_3	18.7	0.3	12.3	ND
M_4	7.3	0.1	9.2	ND

M_1: Mucilage prepared from HDFM by coprecipitate method; M_2: Mucilage prepared from HDFM by modified coprecipitate method; M_3: Mucilage prepared from HDFM by boiling water method; M_4: Mucilage prepared from HDFM by enzymatic method

Table 3: Compositional analysis of mucilage/protein products prepared by four methods from hexane defatted meal detoxified using isopropanol (HDFM- I)

Mucilage /protein product	Protein (%)	Oil (%)	Ash (%)	CG as HCN
M_1	64.9	0.5	9.3	ND
M_2	8.6	0.3	9.8	ND
M_3	19.8	0.3	11.2	ND
M_4	7.8	0.3	9.4	ND

M_1: Mucilage prepared from HDFM by coprecipitate method; M_2: Mucilage prepared from HDFM by modified coprecipitate method; M_3: Mucilage prepared from HDFM by boiling water method; M_4: Mucilage prepared from HDFM by enzymatic method

Dietary fiber content of mucilage/protein products: Table 4-6 gives results of the TDF, SDF and IDF content of the four mucilage/protein products namely M1, M2, M3 and M4 prepared from IDFM, HDFM-MM and HDFM-I flaxseed protein products.

Mucilage/protein product M1 prepared by the co-precipitate method from IDFM (Table 4) can be considered poor in mucilage containing, 22.1% TDF, 15.6% SDF and 6.94% IDF. M2 and M3 are rich mucilage products containing 75.3 and 63.21% TDF, respectively and 73.8 and 56.9% SDF, respectively and 1.5 and 6.3% IDF respectively, M4 although rich in TDF 77.8% yet most of it is ISF 72.6% and thus is very poor in the SDF 5.2%.

Table 5 indicates the TDF, SDF and IDF of four Products M1, M2, M3 and M4 prepared from HDMF-MM. As in previous table mucilage/protein products prepared by different methods from HDFM-MM follow same trend of results as those prepared from IDFM. M1 contains little TDF 20.8, 16.2% SDF and 4.5% IDF. M4 although rich in TDF 77.3%, yet very poor in SDF 6.3% and very high in IDF 71%. M2 and M3 contain high levels of TDF mainly in the soluble form. TDF and SDF are 85.6 and 83.9% for M2, respectively and 65.2 and 58.6% for M3, respectively.

Results in Table 6 follow the same trend as those of Table 4 and 5. Table 6 represents values of dietary fiber content of four mucilage/protein products prepared from HDMF-I. M1, M2, M3 and M4 have 21.8, 86.1, 87.7 and 75.6% TDF, respectively, 16.3, 85.9, 61.1 and 6.8% SDF, respectively and 5.5, 0.18, 6.6 and 68.8% IDF, respectively.

Table 4: Dietary fiber content of mucilage/protein products prepared by four methods from isopropanol defatted flaxseed meal (IDFM)

Mucilage /protein product	Total dietary fiber	Soluble dietary fiber	Insoluble dietary fiber
M_1	22.10	15.16	6.94
M_2	75.30	73.80	1.50
M_3	63.21	56.90	6.30
M_4	77.80	5.20	72.60

M_1: Mucilage prepared from HDFM by coprecipitate method; M_2: Mucilage prepared from HDFM by modified coprecipitate method; M_3: Mucilage prepared from HDFM by boiling water method; M_4: Mucilage prepared from HDFM by enzymatic method

Table 5: Dietary fiber content of mucilage/protein products prepared by four methods from hexane defatted flaxseed meal detoxified with methanol mixture (HDFM-MM)

Mucilage /protein product	Total dietary fiber	Soluble dietary fiber	Insoluble dietary fiber
			4.5
M_1	20.8	16.2	1.7
M_2	85.6	83.9	7.1
M_3	65.2	58.6	71.0
M_4	77.3	6.3	4.5

M_1: Mucilage prepared from HDFM by coprecipitate method; M_2: Mucilage prepared from HDFM by modified coprecipitate method; M_3: Mucilage prepared from HDFM by boiling water method; M_4: Mucilage prepared from HDFM by enzymatic method

Table 6: Dietary fiber content of mucilage /protein products prepared by four methods from hexane defatted flaxseed meal detoxified using isopropanol (HDFM-I)

Mucilage /protein product	Total dietary fiber	Soluble dietary fiber	Insoluble dietary fiber
M_1	21.8	16.3	5.50
M_2	86.1	85.9	0.18
M_3	67.7	61.1	6.60
M_4	75.6	6.8	68.80

M_1: Mucilage prepared from HDFM by coprecipitate method; M_2: Mucilage prepared from HDFM by modified coprecipitate method; M_3: Mucilage prepared from HDFM by boiling water method; M_4: Mucilage prepared from HDFM by enzymatic method

Antioxidant activity of mucilage/protein products: Antioxidants (AO) possess a very important property that is essential for the body to be able to combat excess Free Radicals (FR), which are molecules that may harm the body and may cause cell damage. It seemed important to evaluate the antioxidant activity of the prepared mucilage/protein products.

Figure 1-3 show the antioxidant activity of the four mucilage/protein products M1, M2, M3 and M4 prepared from IDFM, HDFM-MM and HDFM-I, as determined by the ß-carotene/linoleate system and measured by changes in absorbance at 270 nm. Figure 2 indicates that the highest AOA was achieved by M2 followed by M1>M3>M_4 with values of 85.9, 82.5, 80.7, 80.1%, respectively, when prepared from IDFM. Figure 1, shows 79.8, 72.4, 77.6, 70% AOA for M2, M1, M3 and M4, respectively, when prepared from HDFM-MM. While M1, M2, M3 and M4 prepared from HDFM-I (Fig. 3) gave the following values of AOA 82.2, 77.6, 79.7, 72.3%, respectively, These results also show that the mucilage/ protein products prepared from IDFM showed higher levels of AOA than those prepared from HDFM-I followed by those prepared from HDFM-MM. It can be clearly seen from Fig. 1-3, that the four mucilage products (M1, M2, M3 and M4) possess different levels of AOA, also that all the mucilage/protein products were superior to the control (ß-carotene emulsion) in their antioxidant capacity.

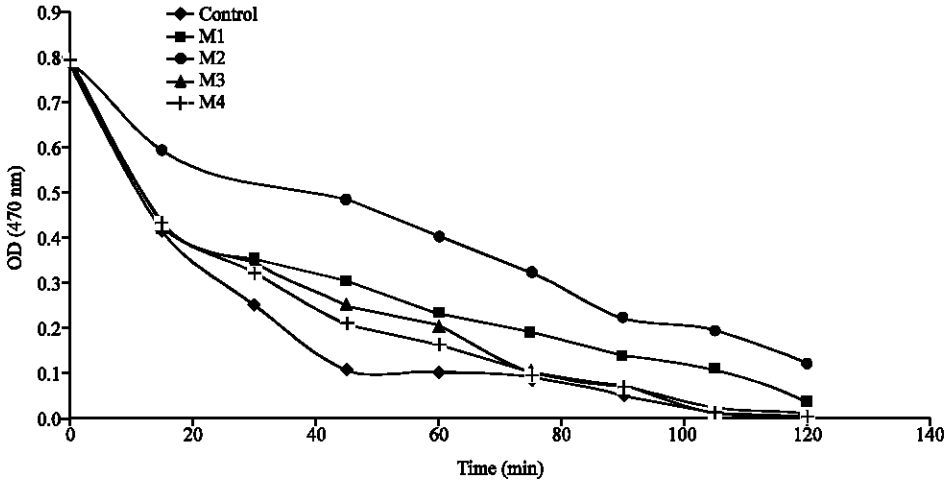

Fig. 1: Antioxidant activity of four mucilage protein products prepared from HFDM-MM in a B-carotene/linoleate system as measured by changes in absorbance at 470 nm

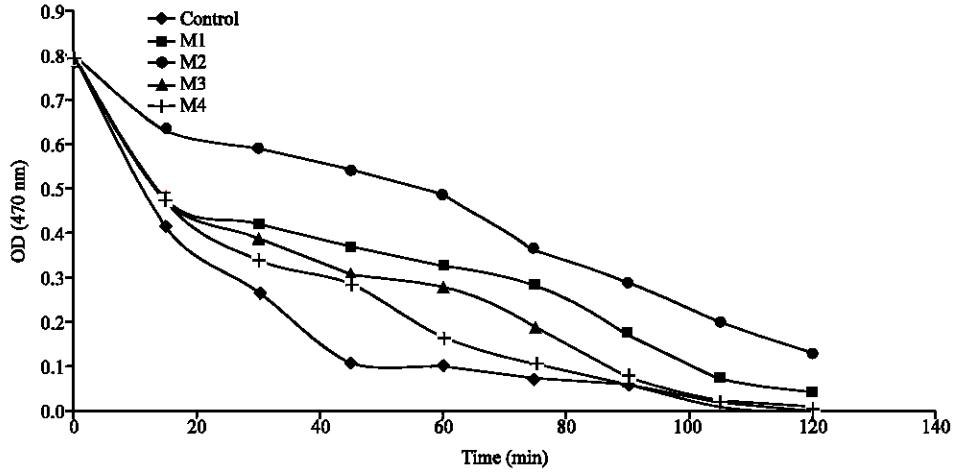

Fig. 2: Antioxidant activity of four mucilage /protein products prepared from IDFM in a B-carotene /linoleate system as measured by changes in absorbance at 470 nm

Effect of different processing conditions on the mucilage yield: As a result of the previous work it can be concluded that defatting flaxseed with isopropanol to give (IDFM) would be advisable, but since the oil industry mostly uses hexane for defatting of prepress flaxseed cake, it seemed more realistic to work on the hexane defatted flaxseed meal (HDFM). Unfortunately HDFM contains CG compounds. Previous results revealed that both detoxification of HDFM with methanol mixture or with isopropanol yielded mucilage protein products with high levels of SDF. There are some restrictions concerning the use of methanol in food, therefore hexane defatted meal, then isopropanol detoxified (HDFM-I) was chosen for further work.

Some of the steps encountered during the preparation of the mucilage/ protein products were further investigated.

Meal to water ratio: In the four methods used for the preparation of mucilage/protein products the first step was to form a meal slurry in water. Investigation of the effect of the Meal: Water ratio

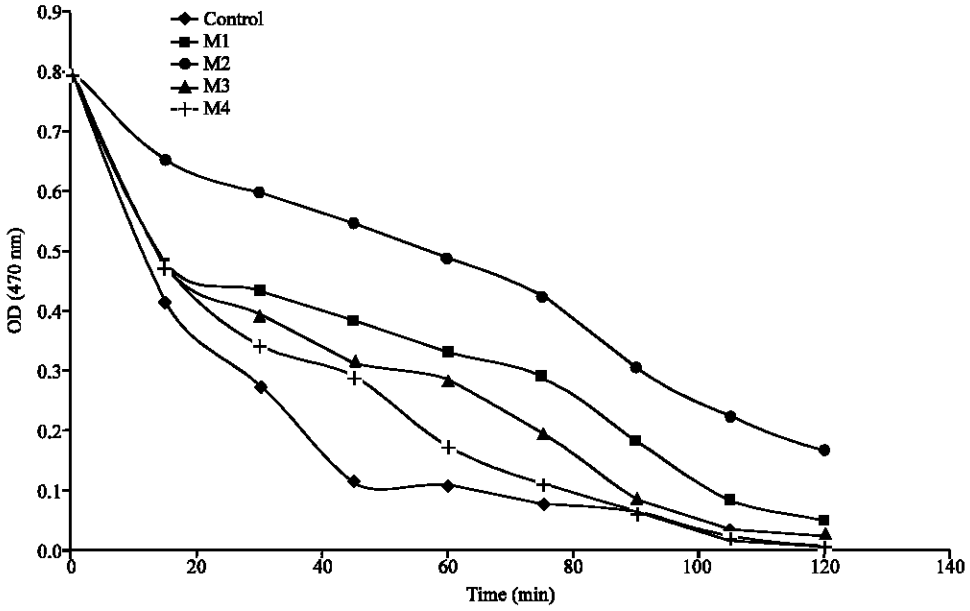

Fig. 3: Antioxidant activity of four mucilage/protein products prepared from HDFM-I by the B-carotene linoleate method as measured by changes in absorbance at 470 nm

(w:v) on the resulting weight of the final mucilage extract (or mucilage yield) seemed necessary. Figure 4 is a diagramatic representation for the effect of meal: water ratio on mucilage/protein products prepared from HDFM-I. It can be clearly seen that the same trend of results was followed for all samples (M1, M2, M3 and M4). The mucilage yield was directly proportional to the increase in Meal: Water ratio until it reached 1:40 where optimum yield was obtained for the four samples.

Centrifugation temperature: The centrifugation temperature during the preparation of mucilage/protein products from HDFM-I was investigated. Figure 5 represents the effect of centrifugation temperature on the mucilage yield obtained from the coprecipitate method, modified coprecipitate, boiling water and enzymatic treatment methods, prepared from HDFM-I. Results in Fig. 5, clearly demonstrate how the centrifugation temperature affects the mucilage yield. Best yield was achieved with the lowest temperature investigated 5°C ,where M1, M2, M3 and M4 resulted in mucilage yield 95, 96, 95 and 95 g, respectively. As the temperature was raised the mucilage yield became less. This applies for the four methods investigated.

Mucilage: Ethanol ratio: The mucilage to ethanol ratio (v/v) was investigated for the two methods in which they were used namely: modified coprecipitate method and the boiling water method, where ethanol was applied to precipitate mucilage. The mucilage: ethanol ratios used included: 1:10, 1:20, 1:30, 1:40, 1:50 and 1:60. Results of this experiment are demonstrated in Fig. 6 where highest mucilage yield was achieved at 1:50 mucilage: ethanol ratio.

Comparing the four methods for the preparation of mucilage products it can be concluded that the coprecipitate method gave a high protein/low mucilage product. The modified coprecipitate method gave a high mucilage/low protein product (M2). The boiling water method resulted in a mucilage/protein product that was very dark in color. Finally the enzymatic method gave a mucilage/protein product that was dark in color and contained very little SDF and mostly IDF.

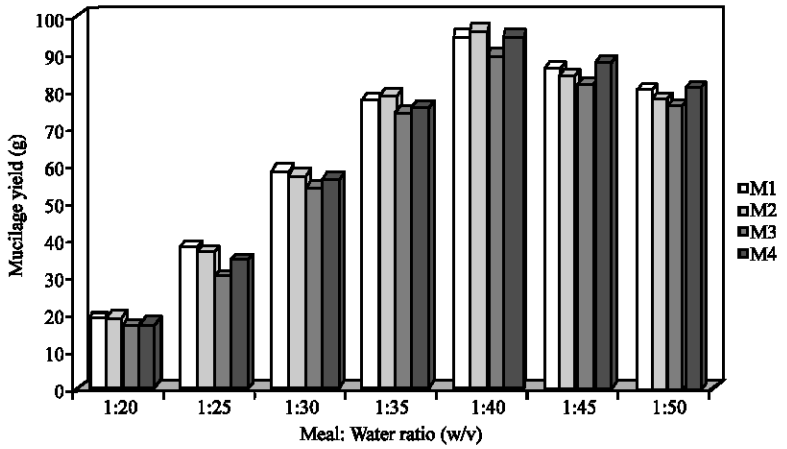

Fig. 4: Effect of meal :water ratio on mucilage yield of the four mucilage/protein products prepared from HDFM-I

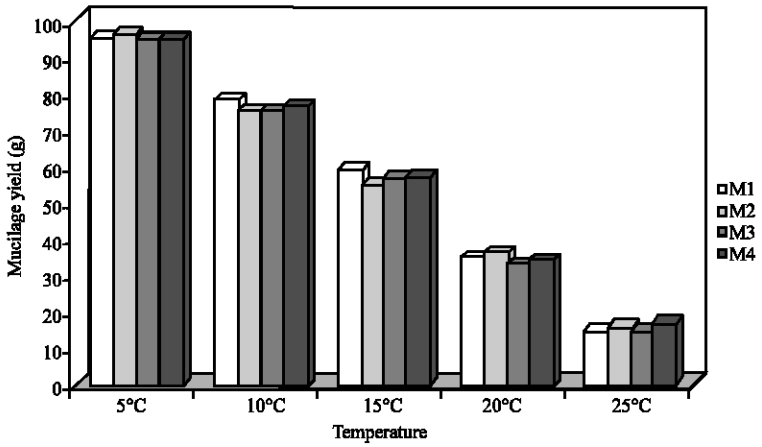

Fig. 5: Effect of centrifugation temperature on mucilage yield of the four mucilage/protein products prepared from HDFM-I

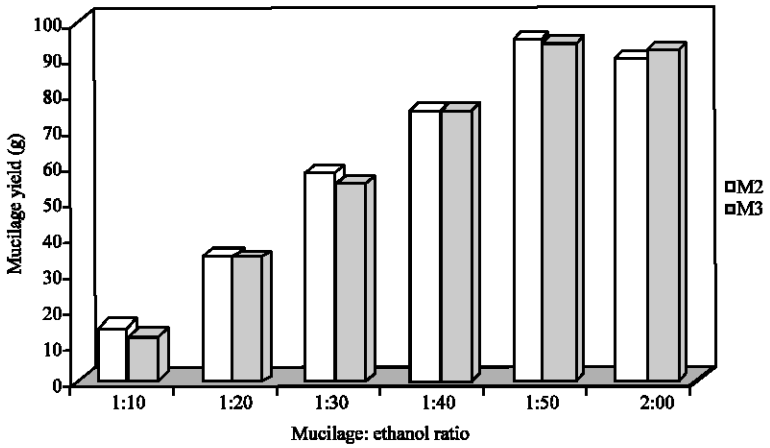

Fig. 6: Effect of mucilage to methanol ratio on mucilage yield of two muculage/protein products prepared from HDFM-I

Table 7: Functional properties of mucilage/protein product (M_2) prepared from hexane defatted flaxseed meal detoxified with isopropanol (HDFM-I) and freeze dried

Functional properties	M_2
Wettability (sec)	45.0
Flowability (sec)	15.0
Bulk density (g mL^{-1})	0.4
Water absorption capacity (WAC) %	600.5
Oil holding capacity (%)	96.5
Emulsifying capacity (%)	95.3
Gelation (%)	11.0
Foam stability (min)	45.0

Therefore, the preparation of a mucilage/protein product is preferably done by the modified coprecipitate method from hexane defatted flaxseed meal then isopropanol detoxified (HDFM-I). The modified coprecipitate method yielded highest mucilage in final product. M2 also it exhibited the highest AOA.Optimum conditions for the preparation of M2 are achieved by using a Meal: Water ratio of 1:40 (w/v) and Mucilage : Ethanol ratio of 1: 50 (v/v) and carry centrifugation at 5°C.

Functional properties of mucilage/protein product (M2): Functional properties of mucilage/protein product M2 is represented in Table 7.

Wetting ability of M2 took 45 sec, flowability 15 sec and bulk density shows 0.4 g mL^{-1}. Water absorption capacity is quite high reaching 600.5%, while oil holding capacity showed a value of 96.5%. Emulsifying capacity of M2 indicated 95.3% and gelation of M2 is 11%. The foam stability of M1 reached 45 sec.

Fiber water product: Water is essential for living. Any human being young or old needs water to survive. Water would be the most simple and suitable vehicle to carry the dietary fiber needed for healthy living to people and animals. Thus water was fortified with 0.01, 0.03 and 0.05% mucilage/protein product M2 and filled in bottles that were thermally processed in boiling water for 20 min, sealed with screw caps and stored in refrigerator at 6±2°C.

Microbiological examination of fiber water: Total viable bacterial, spore forming bacteria, coliform, yeasts and molds were not detected in all water samples during storage.

Sensory evaluation of drinking water prepared with different concentrations of mucilage: Figure 7 is a diagrammatic representation of the mean values for sensory evaluation of drinking water samples prepared with different concentrations of mucilage at the beginning of storage, where it is clear that panelist gave a 10 score for parameters investigated. Results reveal that at the end of three months of storage (Fig. 8) color of all samples including control was not changed during storage. From the data, it is also clear that there is no significant difference of color and consistency mean values during storage. The mean values of control samples, samples prepared with 0.01, 0.03 and 0.05% mucilage decreased both from 10, 10, 10 and 10 at the beginning of storage to 9.5, 9.4, 9.4 and 9.4, respectively for odor and to 9.9, 9.9, 9.9 and 9.9, respectively, for consistency at the end of storage. On the other hand, the taste and overall acceptability mean

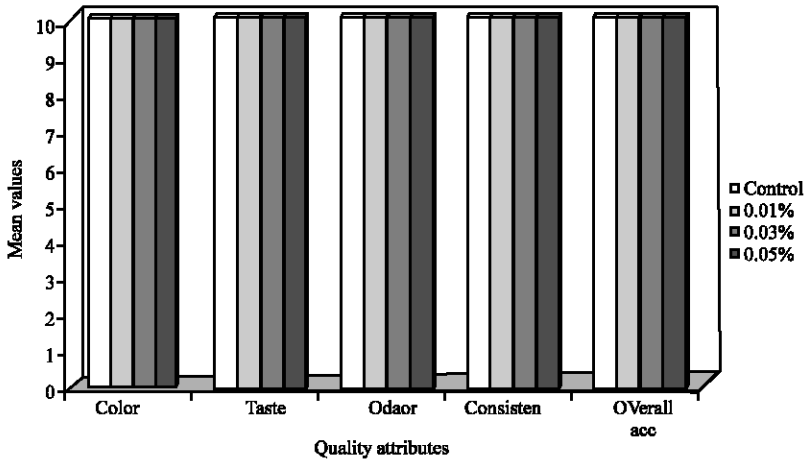

Fig. 7: Mean values of sensory evaluation of fiber water containing different concentrations of mucilage at the beginning of storage

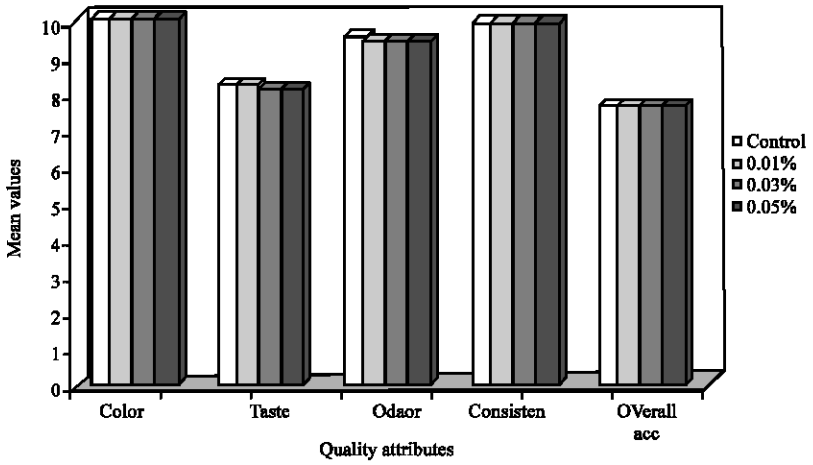

Fig. 8: Mean values of sensory evaluation of fiber water containing different concentrations of mucilage after three months of storage

values of control samples and samples prepared with 0.01, 0.03 and 0.05% mucilage were significantly decreased both from 10, 10, 10 and 10 at beginning of storage to 8.2, 8.2, 8.1 and 8.1, respectively for taste and to 8.0, 8.0, 8.0 and 8.0, respectively, for overall acceptability at the end of three months storage.

DISCUSSION

The preparation of the mucilage/protein products by the four different methods namely, coprecipitate method, modified coprecipitate method, water boiling method and enzymatic method (Table 1-3) reveal that all the M1 products prepared by the coprecpitate method were products rich in protein ranging from 63- 65% and are considered protein concentrates. Dev and Quensel (1988) used the same procedure and extracted protein and mucilage from the isopropanol defatted flaxseed meal in NaOH solution pH 9.5-10 and coprecipitated at pH 4.2-4.5 using HCl following centrifugation the precipitate was neutralized and spray dried. They obtained a high mucilage

protein product containing 63.4% crude protein.. Their results are in agreement with our results. Probably precipitating the alkaline solution containing both protein and mucilage at the IEP of the protein is not an efficient way to precipitate all the mucilage.

M2, M3 and M4 are products containing little protein. All M2 products prepared by the modified coprecipitate method contains little protein probably because the mucilage was precipitated from the alkaline extract using ethanol which is efficient for precipitating mucilage and not the protein. This result is confirmed by the work of Kankaanpaa-antilla (1999) who suggested in their patent the use of an acid and an alkanol to precipitate proteins and mucilage from the alkaline extract. The alkaline extract containing protein and mucilage is centrifuged, then the acid is added to precipitate the protein at its IEP. The ethanol can be added directly to the acidified mixture, or the acidified mixture can be first separated into a precipitate fraction and a filtrate fraction, whereby the ethanol is added to the filtrate. The alcohol can be added simultaneously with the acid, or the alcohol precipitation can be performed wholly before the acid precipitation. In this case 60 to 90% of the dissolved pentosans are recovered. As for products M3 which are prepared by the water boiling method, they all contain between 19-20% protein which represents the protein fraction extracted at the pH of the water about (pH 6.5). The enzymatic method for preparation of mucilage/protein M4 gave products very low in protein content about 7.8% protein. This is expected because the hydrolysis step using the protease enzyme hydrolysis and removes most of the protein. Same result was reported by Holmgren (1990) who patented an enzymatic hydrolysis method for the preparation of a mucilage product with low protein content. The preparation of the mucilage protein products from different starting materials namely IDFM, HDFM-MM and HDFM-I did not have significant effect on the composition of the products. It is worth mentioning that all prepared mucilage/protein products were free of CG.

Coming next to the dietary fiber content of products M1, M2, M3 and M4 prepared from different treated flaxseed (Table 4-6) It can be clearly seen that as expected products M1 that contain high protein, contain less TDF than the other products M2, M3 and M4. Dev and Quesel (1988) following the same procedure reported product containing high protein 63.4% and total pentosans 9.13%, soluble pentosans 7.34%. Pentosans reflect the amount of polysaccharides in food fiber. Results indicate that nowhere near all of the polysaccharides were precipitated by the method. Wanasundara and Shahidi (1997) also removed gums by alkaline extraction. Our results also show that most of TDF is in the form of SDF 15-16% SDF and only 5-7% ISF. In our opinion precipitation of the alkaline mucilage-protein extract at IEP of the protein precipitates mostly proteins. Mucilage/protein products M2 prepared by the modified coprecipitate method which comprises alkaline extraction of both mucilage and protein then precipitating the mucilage in ethanol, revealed high content of TDF 75-86%. The SDF comprised between 98 - 99.8% of the TDF. Mucilage /protein product prepared by this method resulted in product M2 with highest content of SDF and low protein content, probably the ethanol precipitates mostly the mucilage but protein. This result is in agreement with Kankaanpaa-antilla (1999) who patented a process where the precipitation of the mucilage using an alkanol was recommended. Mazza and Oomah (1995) reported the precipitation of mucilage with ethanol then freeze drying. The extraction of the mucilage with hot water then precipitation with ethanol resulted in Mucilage/protein products M3 that reveal high TDF content 63-67% which are mostly in the soluble form 56-61% SDF, the rest was in the insoluble form. Although, M3 is a high mucilage-low protein product, yet we encountered problems during filtration and the product was rather dark. Some of the authors reporting on the extraction of mucilage or gum with hot water include (Mazza and Billiardis, 1989;

Bhatty, 1993; Cui *et al*., 1994b; Thakur *et al*., 2009). The last method comprising the enzymatic hydrolysis of protein and then the carbohydrates yielded high mucilage-low protein products M4 that were unfortunately rich in TDF 75-77%, yet was very poor in SDF 5-7%. Almost all the dietary fiber in the product was in the insoluble form 69-72% IDF. Thus this procedure can be recommended for the preparation of dietary fiber product which is high in IDF. Holmgren (1990) and Yunosov and Steven (2009) also reported on the sequential use of different enzymes, for the preparation of mucilage product from several seeds. It should be mentioned that the type of detoxified flaxseed meal used for the preparation of Mucilage/protein products did not have much influence on the dietary fiber content of the products.

Results of the antioxidant activity of the four mucilage/protein products by the β-carotene /linoleate method and measured as changes in absorbance at 470 nm revealed that all the mucilage products possess AOA which is higher than the control. M2 prepared from IDFM (Fig. 2), exhibited highest AOA reaching 85.9%, generally M2 possessed the highest AOA when products were prepared from HDFM-MM (Fig. 1) and HDFM-I (Fig. 3).

Probably this result is due to the ability of methanol to extract more phenolic compounds than isopropanol. The antioxidant activity of flaxseed is attributed to its content pf phenolic compounds. Taha *et al*. (2003) and Strandas (2008) reported on the presence of phenolic compounds in flaxseed. Many authors attributed the AOA of dietary fiber products to the presence of phenolic compounds (Larrauri *et al*., 1997). In agreement with our results. Taha *et al*. (2003) reported antioxidant activity coefficient of SDF and IDF of different flaxseed protein products. But contrary to our results they found IDF fraction to possess more AOA than SDF fraction. Also contrary to our results Velioglu *et al*. (1998) investigating the AOA of flaxseed among other vegetables and grains and reported that flaxseed gum which was not precipitated with 95% ethanol, showed strong AOA of 78.6% because of its high phenolic content. In our work the mucilage product precipitated with ethanol showed higher AOA than the other products. The AOA of dietary fiber products is well documented (Saura-Calixto, 1998; Jiminez-Escrig *et al*., 2001; Liobera and Cañellas, 2008).

Flaxseed meal HDFM-I was chosen as the starting material as explained in the results part. It then became important to investigate the effect of different processing conditions on the mucilage yield. Results of this investigation indicated optimum conditions for the preparation of M2 was achieved by using a Meal : Water ratio of 1:40 (w/v) and Mucilage:Ethanol ratio of 1: 50 (v/v) and carry centrifugation at 5°C. Under the previous conditions, M2 was chosen to be prepared for further work. M2 was mainly chosen for further work as it has the highest SDF and highest AOA of the Mucilage/protein products. Some functional properties of M2 prepared from HDFM-I is represented in Table 7.

Wettability means the ability of a powder to be wetted. It is expressed as the time in seconds a certain quantity of powder needs to penetrate into a calm surface. It is important for instant products. M2 took rather much time to get wetted (45 sec), compared to soybean meal (16 sec) (Taha and Ibrahim, 2002). Flowability signifies the ability of a powder to flow, it is also an important criteria for instant products. Flowability of mucilage/protein product M2 is achieved in 15 sec. while soybean meal took 30 sec (Taha and Ibrahim, 2002). This means M2 has good flowability. In order to choose appropriate packaging units it is advisable to determine bulk density of any instantised product. Bulk density was 0.4 g mL^{-1} compared to 0.625 g mL^{-1} for soybean (Taha and Ibrahim, 2002). Thus it needs small packaging units. Water absorption capacity is the ability of a product to absorb water or swell. It is important in the manufacture of bakery products, pastas, doughnuts and others. Consumers tend to avoid products that show free water in the

package, thus the importance of the water holding capacity. It also determines the acceptability of a given food regarding texture, juiciness and mouth feel. Water absorption capacity of M2 is 600.5% compared to 300% for soybean meal (Taha and Ibrahim, 2002) and 585% for flaxseed meal (Taha *et al*., 2003). Dev and Quensel (1988) reported high mucilage protein products to absorb higher amounts of water 419-470% than low mucilage protein products 313-141%. Fedeniuk and Billiardis, 1994), determined the water binding capacity of three flaxseed gums by the Baumann capillary apparatus and found it between 1600-3000g H_2O/100 g solids. Wanasundara and Shahidi (1994) attributed the high water absorption capacity of flaxseed meal to be mainly to the presence of the polysaccharides (mucilage) in the seed coat (Fedeniuk and Billiaderis, 1994). Oil holding capacity is the ability of a product to bind with oil. It is important in the meat industry (sausages,hamburgers etc. and also in sometimes in the bakery industry e.g., Doughnuts. As the water holding capacity they not only determine the acceptability of a given food product but also the profit margin. Formulations that result in poor water and oil holding capacity translate to liquid losses during processing (cooling, freezing etc). Oil holding capacity is found to be. Dev and Quencel (1988), reported 95% oil absorption for high mucilage flaxseed protein concentrates and 141% for low mucilage protein concentrate. Emulsifying and film forming ability of protein products is essential to perform well in meat systems also the ability to form emulsions is critical to their application in mayonnaise, salad dressing, milks and frozen deserts. Results in Table 7 show emulsifying capacity of M2 to be 95.3%. Dev and Quencel (1988) reported 96 and 98% for high mucilage protein concentrates and 51% for low mucilage protein concentrate from flaxseed. Dev and Quencel (1988), Mazza and Billiardes (1989) reported that gum in flaxseed has been implicated in enhancing viscosity, water binding, emulsifying and foaming properties in linseed. Gelation is important in comminuted meat products as emulsifying capacity. It is reported as the lowest concentration of product that remained as a stable gel after 30 min. Results show gelation of M2 is 11%. High mucilage flaxseed protein concentrate and low mucilage flaxseed protein concentrate were 12% as reported by Dev and Quencel (1988). Chen *et al*. (2005), Cui *et al*. (1994a) reported that flaxseed gum exhibits weak gel- like properties that can be used to replace most of the non gelling gums for food and non food applications. Chen *et al*. (2005), also studied the gel formation and the effects of different factors on the gel strength of flaxseed gum. Their study indicates that flaxseed gum solutions were characterized with gelation that could form a thermoreversible cold set gel. The gelling and melting points of flaxseed gum were influenced by the initial cooling temperature and the gelling temperature was lower than its corresponding melting point.

Foam stability is the capacity to form stiff stable foam and is an important requirement of products to be incorporated in whipped toppings, gel cakes and soufflé like products. Foam stability of M2 reached 45 sec. Dev and Quencel (1988) reported foaming capacity of high mucilage flaxseed protein concentrate to be 226 and 166% and foam half life to be 50 min and for low mucilage protein concentrate to be 10% and 70 min for foam capacity and foam half-life, respectively. Mazza and Billiardis (1989) studied the foam capacity and stability of aqueous dipersions of flaxseed gum and were determined as a function of concentration and compared the results to the data obtained for ovalbumin at the same concentrations. For 1% (w/v) solutions flaxseed gum gave ca. 75% of those of ovalbumin. And has similar time-dependant stability. With less concentration foam values decreases and are less stable.

Results of the functional properties examination indicate that Mucilage/ protein product M2 has poor wettability poor gelling properties, reasonable flowability, oil holding capacity, good emulsifying property and Foam stability and very good water absorption capacity.

Fiber water samples fortified with 0.01, 0.02 and 0.05% M2, did not show any microbiological contamination after 3 months of storage. This can due to the good quality of tap water that resulted from residual chlorine used for disinfecting pumped tap water and also because of insufficient nutrients in tap water as well as the possible antimicrobial activity of mucilage in the samples prepared with it and the cold storage. Sensory evaluation of the fortified fiber water, as well as control after three months of storage did not show any changes in color nor consistency of the water, while the odor was slightly affected, it scored (ca.9). On the other hand, taste and overall acceptability were more affected still, they scored (ca.8).

This fiber water can also be used to cook eg. rice, oatmeal and others without changing their taste, prepare beverages, tea or coffee to supplement the body with the soluble fiber it contains. Several authors have patented methods for the preparation of fiber water or water supplemented with fiber and other healthy ingredients. Stillman (2001, 2007), also patented a process for the preparation of fiber-water, water containing soluble fiber, which was shelf stable, ready to use, essentially tasteless and odorless water-like fluid for humans and animals. Hasting (1996) patented an invention involving a beverage composition that supplies fiber, herbs, antioxidants and enzymes to the human body. Gandi (1998) patented a formulation of physiologically-effective clear/translucent beverage containing non-gel forming soluble fiber and a soluble salt of calcium. It is worthwhile to mention here that non of the former authors used flaxseed mucilage as the soluble dietary fiber source. The trend of fortified water started in Japan yet it became afterwards of much interest in United States, Europe and beyond. In Japan it is called near water and examples are water containing vitamins, reishi, seaweed extract and chamomile. In other countries it is also called enhanced water and functional water. No water containing mucilage is yet found in the market.

CONCLUSION

In conclusion, the preparation of a high mucilage low protein product (M2) from detoxified flaxseed meal by the modified coprecipitate method is highly recommended. Optimum condition for its preparation include: the use of 1:40 meal: water ratio, 5°C and 1:50 mucilage: ethanol ratio. It exhibited good AOA and was successfully incorporated in a water product Fiber Water which can act as a vehicle to supply any age group with the needed soluble fiber. M2 can be used to fortify bakery products, pastas etc., also in meat systems, mayonnaise, salad dressing and whipped toppings, soufle due to its good emulsifying property and foam stability, respectively.

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