

DIETARY FIBRES COMPOSITION ANALYSIS OF CAROB SEED TEGUMENT AS LOCUST BEAN GUM CONTAMINANTS'

**Patrick Aubin Dakia^{1,2*}, Marie-Michel Agnan Combo³, Grodji Albarin Gbogouri²,
Kouakou Brou², Michel Paquot¹**

¹University of Liège – Gembloux Agro-Bio Tech, Unit of Biological and Industrial Chemistry (CBI), 2, Passage des déportés, 5030 Gembloux, Belgium

²University of Nangui Abroguoa (ex Abobo-Adjame), Department of Food Science and Technology, 02 BP 801 Abidjan 02, Tel: +225 47492461, Abidjan, Côte d'Ivoire

³University of Jean Lorougnon Guede, Department of Agroforestry, Biochemistry and Microbiology, BP 150 Daloa, Côte d'Ivoire

ABSTRACT: *The proximate composition and the fibres content of the carob seed tegument were analyzed in order to evaluate the effect of carob seed hull fragments (as contaminants) on locust bean gum (a galactomannan usually used as food additive) composition. The separation of the seed components by boiling water pre-treatment furnished ~30% of brown coat. The seed coat fibres analysis by enzymatic-gravimetric method with phosphate buffer showed that carob husk contain ~75% IDF (insoluble dietary fiber) and ~15% SDF (soluble dietary fiber). In addition, the NDF (neutral-detergent fibre) composition, determined by non-enzymatic-gravimetric method with detergent solution, revealed the presence of ~20% of insoluble hemicelluloses, ~33% of cellulose and ~9% of lignin fractions. These results suggest that this product may be regarded as a potential fibre source for Locust bean gum flour enrichment, and suitable for use as food ingredient.*

KEYWORDS: Ceratonia siliqua L., Dietary fibre, Carob seed Tegument, hull, husk, Locust bean gum,.

INTRODUCTION

Carob tree (*Ceratonia siliqua* L.), a long-lived evergreen tree, has an economic and environmental importance around Mediterranean area. The tree is used in reforestation of arid areas. The pulp and the seeds in the carob fruit pod have some interesting properties and are often valorized in different applications in food and pharmacological industry (Batlle and Tous, 1997; Kivrak et al., 2015; Haddarah et al., 2013, 2014; Bouzouita et al., 2007; El Batal et al., 2013).

Carob pods pulp, used as pet food and chocolate powder substitute, is characterized by high sugar content (500 g/kg) and contain appreciable amount of fiber (29-39%), depending on the type of the extracted fiber (Batlle and Tous, 1997; Sigge et al., 2011). Hariri et al. (2009) reported that the pod fiber content play a role in hypocholesterolemic and hypoglycemic regulation,

Published by European Centre for Research Training and Development UK (www.eajournals.org)

Carob seed endosperm powder called locust bean gum (LBG, additive E 410) is used as stabilizer and thickening agents in food and in pharmaceutical industries (Batlle and Tous, 1997; Neukom, 1989; Dakia et al., 2010). It can be used in baby foods to prevent vomiting (Sandolo et al., 2007, Dakia et al., 2011). Carob seed endosperm is composed of reserve polysaccharides (hemicelluloses) called galactomannans which consist of α -(1 \rightarrow 4)-D-mannopyranosyl backbone substituted to varying degrees at -(1 \rightarrow 6) with single-D-galactopyranosyl residues (Dea, 1975; Dakia et al, 2008; Gillet et al., 2014, 2017)

The seed is composed of the tegument (30-33%), the endosperm (39-46%) and the germ (17-25%) (Neukom, 1988; Dakia et al, 2008, 2017a). The first stage of the endosperm (LBG source) extraction involves removal of the tight-fitting brown coat; either by thermo-mechanical or by chemical treatment. In all procedures for carob gum extraction, it is difficult to avoid the presence of impurities from hull pieces in crude LBG flour (Hefti, 1957; Da Sylva et al, 1990; Ensminger et al., 1994; Kivrak et al., 2015; Dakia et al, 2007; 2008, 2017a, 2017b, 2018).

Dakia et al (2017b) had compared the monosaccharide's composition of carob seed tegument with locust bean gum hot-water-insoluble residue, and suggested that the minor sugars, particularly arabinose and xylose (non-galactomannan polysaccharides), usually determined during LBG flour analysis, originate (theorically from pectin and hemicellulosic polysaccharides) from hull fractions remained in the carob gum flour during its primary extraction process.

Pectin, hemicellulose, gums, β -glucans, cellulose and lignin are included in Dietary fibre which consists of a variety of non starch polysaccharides and associated substances, resistant to digestion (hydrolysis) by human alimentary enzymes (Prosky, 2000 ; Lamghari et al., 2000 ; Gallaher and Schneeman, 2001).

However, in spite of the great interest to carob and their use in different applications, there is no literature data on carob seed hull fibres content.

So the purpose of this study was to analyze the dietary fibre content of carob seed husk in order to use it as a potential fibre source in the enrichment of foods. The composition of the different fibre fractions was determined by enzymatic and non-enzymatic gravimetric methods.

MATERIAL AND METHODS

RAW MATERIALS

In this study, the carob seeds used (length: 5.5-6 mm x thickness 3.5-4 mm) were obtained from T.A.S.A., Parque Arboretum de Algarrobos (Malaga, Spain). The carob seed hull was obtained after an aqueous thermal pre-treatment of carob seeds under the following conditions: 100 g (~780 seeds) of whole seeds immersed in 800 ml of boiling water during 60 min. The swelled seeds, without tegument disruption, were then easily separated manually to obtain the husk, endosperm and germ components. A pure flour of seed coat (hull, testa) was then obtained by drying (100°C/30 min) and milling (IKA A10) the hull fraction.

Proximate composition

Moisture content of samples was determined by oven-drying, using an air-circulated oven at 106 °C, for 24 h. All values were calculated on a dry-weight basis. The *ash* content of the carob gum was determined gravimetrically after dry mineralization at 600°C for 12 h. *Lipids* content were determined by using chloroform/methanol (2/1 v/v) mixture, as described by Folch, Lees and Stanley (1957). *Protein* content was determined by the Kjeldahl method (AOAC, 1984), after mineralization with a Digestion System 20 (Tecator AB, Höganäs, Sweden) and distillation by a Kjeltex Auto 1030 Analyser (Tecator AB, Höganäs, Sweden).

Dietary fibre composition analysis

Dietary Fibre by Enzymatic-Gravimetric Method (AOAC 991.43)

Total, Soluble, and Insoluble Dietary Fibre were determined by the enzymatic-gravimetric method, with MES-TRIS Buffer according to AOAC 991.43 method (ref. JAOAC 75, May/June issue (1992), described by Lee et al. (1992). Note that, there is no substantiated evidence that there is any significant difference in TDF values as measured by the two dietary fiber analytical methods (AOAC 985.29/AACC 32-05 and AOAC 991.43/AACC 32-07, “old” with Phosphate buffer and “new” with MES-TRIS buffer, respectively) over all foods. Minor (subtle) changes have been made to reduce analysis time and to improve assay precision in 991.43 (Lee et al., 1992; Haralampu, 2000; Prosky et al., 2001).

Principle

Lee et al. (1992) method extract lipid (for product with Lipid > 10%), digest starch and protein enzymatically, and arrive at the remaining non-digestible fiber content gravimetrically as follow: Duplicate samples; undergo successive enzymatic digestion by heat stable α -amylase, protease, and amyloglycosidase to remove starch and protein. For total dietary fiber (TDF) determination, all enzyme digestate solution is directly treated with alcohol (fibres that are dispersible in water) before filtering, and TDF residue is weighed. However to obtain insoluble and soluble dietary fiber (IDF and SDF), enzyme digestate is first filtered (to separate the IDF from the SDF), and residue (IDF) is dried and weighed. Then the combined filtrate and washes, previously recovered from IDF determination, are treated with alcohol (to precipitated SDF), filtered, dried, and weighed. TDF, IDF, and SDF residue values are corrected for protein, ash, and blank.

Samples Preparation and Enzymatic Digestion

2 blanks/assay were runned with samples to measure any contribution from reagents to residue. Duplicate 1 g dried (at 60°C/12h) samples, were weighted into 800 mL tall form beakers. 40 mL MES/TRIS buffer solution, pH 8.2, was added to each. Solutions were stirred on magnetic stirrer until sample is completely dispersed (to prevent lump formation, which would make material inaccessible to enzymes).

Then 50 μ L heat-stable α -amylase solution (Termamyl 300L, Cat. No. 361-6282, Novo-Nordisk, Bagsvaerd, Denmark) were added, stirring at low speed. Beakers were covered with Al foil, and incubated in 95-100°C H₂O bath 15 min with continuous agitation. Timing was started once bath temperature reaches 95° (total of 35 min is normally required). All beakers

Published by European Centre for Research Training and Development UK (www.eajournals.org)

were removed from bath, and cool to 60°C. AL foils were removed. Beaker walls was scraped with spatula and rinsed with 10 mL H₂O.

Then 100 µL protease solution (*Cat. No. P 3910*, Sigma Chemical Co., Prepare 50 mg/mL enzyme solution in MES/TRIS buffer fresh daily. Store at 0-5°C) was added to each beaker. Beakers were covered with Al foil, and incubated 30 min at 60° ±1°C with continuous agitation. Timing was started when bath temperature reaches 60°C. Foils were removed. 5 mL 0.561N HCl was dispensed into beakers while stirring. pH was adjusted to 4.0-4.7 at 60°C, by adding 1N NaOH solution (or 1N HCl solution). (Note: It is important to check and adjust pH while solutions are 60° because pH will increase at lower temperatures. At the other hand, pH of blank must be checked routinely as a precaution; and if outside desirable range, samples must be checked also.).

Then 300 µL amyloglucosidase solution (*Cat. No. AMG A9913*, Sigma Chemical Co., Store at 0-5°) was added while stirring. Beakers were covered with Al foil, and incubated 30 min at 60° ±1°C constant agitation. Timing was started, once bath reaches 60°C.

C. Determination of Insoluble Dietary Fiber (IDF)

Enzyme digestate, from enzymatic digestion, was directly filtered through a previously dried (105°/12h) and tared crucible containing 0.5-1g celite bed (*Diatomaceous earth.-Acid washed - Celite 545 AW*, No. C8656, Sigma Chemical Co.). Beaker (800 mL) was rinsed and all remaining particles were quantitatively transferred to crucible using wash bottle with 78% EtOH and rubber spatula. The filtration was conducted into filtration flask using Fibertec vacuum system (Tecator AB, Höganäs, Sweden). Note that when fiber is filtered, Celite separates fiber from fritted glass of filtering crucible (40-60 µm pore size, pyrex 60mL), allowing for easy removal of crucible contents.

Then residue, remained by celite bed in the crucible, was successively washed with 70°C H₂O (~10 mL), and then with ~15 mL portions of 78% EtOH and 95% EtOH. Crucible containing dietary fiber residue and Celite was dried overnight in 105°C oven, cooled in desiccator and weighted. The residue (Fibre + remain protein + ash) weight was calculated by subtracting weight of dry crucible with Celite. One duplicate from each sample was used to determine protein. For ash analysis, second duplicate was incinerated 2 h at 500°C, cooled in desiccator, and weighted. Weight of crucible and Celite were subtracted, to determine ash weight. IDF is weight of residue less weight of protein and ash.

Filtrate and water washings combined, from IDF determination, were transferred to 600 mL tall form beaker, and reserve for determination of fibres that are dispersible in water; namely soluble dietary fiber (SDF).

D. Determination of Soluble Dietary Fiber (SDF)

Four volumes of 95% ethanol are added to the combined filtrate and water washings to precipitate SDF at room temperature overnight. The precipitate is quantitatively transferred and filtered through crucible using vacuum, washed with 78% ethanol, and 95% ethanol successively, and then dried and weighed. The residue weight was calculated. One duplicate is analyzed for protein, and the other is incinerated 2h at 500°C for ash. SDF is weight of

Published by European Centre for Research Training and Development UK (www.eajournals.org)
residue less weight of protein and ash.

E. Determination of Total Dietary Fiber (TDF)

Total dietary fiber can be determined either by summing IDF and SDF, or by independent analysis, as follow:

To each digested sample, four volume of 95% EtOH preheated to 60°C was firstly added to known volume of enzyme digest. The precipitate was allowed to form at room temperature for ~1 h. Alcohol-treated enzyme digestate (or precipitate from enzyme digest) was quantitatively transferred and filtered through a previously dried (105°/12h) and tared crucible containing 1g celite bed as previously described.

Crucible containing dietary fiber residue and Celite was dried overnight in 105°C oven, cooled and weighted. The residue weight was calculated. Duplicates were used to determine protein and ash. TDF is weight of residue less weight of protein and ash.

Dietary Fibre by Non-Enzymatic-Gravimetric Method (AOAC 920.86)

Insoluble hemicellulose, cellulose and lignin were determined by the non-enzymatic-gravimetric method, to AOAC 920.86/AACC 32.10 method, described by Van Soest and Wine (1967).

A. Principle

Reflux degradation/extraction was conducted on the samples in neutral detergent for 90 minto remove starch, proteins, lipids, and soluble fibres (as pectin and soluble hemicelluloses). For Neutral Detergent Fiber (NDF) determination, all chemical digestate solution is filtered through crucible using vacuum, and NDF residue (containing insoluble Hemicullose, cellulose and lignin) is weighed. To obtain Acid-Detergent Fiber (ADF) (containing cellulose and lignin), samples were treated with Acid-Detergent to remove starch, protein, lipid, and pectic and hemicelluloses substances, and then filtered, dried, and weighed. Then, to obtain Acid-Insoluble Fiber (AIF) (containing lignin), the residue (ADF) is treated with sulphuric acid 72% for 3h to remove celluloses substances and then filtered, dried, and weighed. NDF, ADF, and AIF residue values are corrected for ash.

B. Preparation of Neutral Detergent Fiber (NDF)

0.5-1 g sample, ground to pass 1 mm screen, were weighed into a suitable container (250 mL fritted flask) for refluxing. 100 ml acid-detergent solution (~ 100 ml ND/1g sample) and ~0.5 g sodium sulfite with a rubber spatula were added. The solution was then heated to boiling; and samples were refluxed for 90 min. Boiling was adjusted to an even level, to reduce foaming. Sample was filtered on a previously dried (furnace-dried 500°C/2h, let cooled and then oven-dried at 105°C/12h) and tared crucible, using light suction. Residue was washed twice with hot water (90-100° C) and twice with EtOH 95%; and then dried at 100°C for 8 h (or overnight), cooled in a desiccator, and weighed. Yield of recovered neutral detergent fiber was reported as cell-wall constituents or as crude dietary fiber. Noncell-wall material was estimated by subtracting this value from 100. Ash was determined by incinerated crucible 2 hr at 500°C. Ash content was reported of neutral-detergent fiber.

C. Preparation of Acid-Detergent Fiber (ADF)

0.5-1 g sample, ground to pass 1 mm screen, was weighed into a 250 mL fritted flask for refluxing apparatus. 100 ml acid-detergent solution was added for 1g of sample weighed. Sample was heated to boiling and heat was reduced to limit foaming. Samples were refluxed for 90 min and filtered on a previously tared crucible, using light suction (vacuum admitted until/after crucible has been filled). The filtration residue was washed twice with hot water (90-100° C) and twice with EtOH 95%; and then dried at 100°C 8 h or overnight, cooled in a desiccator, and weighed. Yield of recovered acid detergent fiber (ADF) was reported as cellulose and lignin content

D. Isolation of Acid-Insoluble Lignin (AIL)

Crucible was placed in a 50 ml beaker for support. An amount of about halfway (3 cm high) with 72% H₂SO₄ was added to the crucible containing the acid-detergent fiber (ADF) and stirred with a glass rod after 10 min. Crucible with glass rod remain in crucible was let at ambient temperature for 1h. Thus, at hourly intervals (as acid drains away), the crucible was refilled with 72% H₂SO₄, and stirred. After 3 h, the crucible containing the acid-detergent fiber (ADF) was filtered with vacuum, and washed with hot water until free from acid. Stirring rod was also rinsed and removed. Crucible was dried at 103°C and weighed after cooling in a desiccator. Then, crucible was ignited in a muffle furnace at 500°C for 2 h. Crucible was placed when still hot into desiccator, cooled and weighed. Yield of recovered acid insoluble lignin was reported as lignin fraction

The content of cellulose and hemicellulose can be calculated from the contents of NDF, ADF and lignin, as follow: Hemicellulose is weight of NDF residue less weight of ADF residue, while Cellulose is weight of ADF residue less weight of lignin.

All values were determined at least in duplicate and standard deviation (SD) was calculated.

RESULTS AND DISCUSSION

Carob seeds tegument proximate composition

Table 1 shown the results obtained from carob seed hull investigation. Total yield of the brown coat obtained, from the seed constituents' separation after boiling water pre-treatment, accounted for ~30 g/100 g while off-white endosperm accounted for ~50 g/100 g and the yellow germ for ~20 g/100 g, on dry weight basis. The chemical feature of the carob seed hull shown that the protein (3.71 g/100 g DM), lipids (0.54 g/100 g DM), ash (2.34 g/100 g DM) and moisture (0.12 g/100 g DM) content were low. Therefore, the nitrogen free extract determined as total carbohydrate, shows that hull contain a higher content of carbohydrate (93.29 g/100 g DM).

Table 1: Composition of carob seed husk (g/100g on dry matter (DM))

	Carob seed husk
Yield (g/100g DM)	30 ± 0.81
Moisture	0.12 ± 0.90
Ashes	2.34 ± 0.21
Total proteins	3.71 ± 0.42
Lipids (neutral et polar)	0.54 ± 0.70
Nitrogen free extract included fibres	93.29 ± 0.55

All values were determined in triplicate (Means \pm SD). All measurements on a dry wt. basis

Carob seed tegument dietary fibre composition

Dietary fibre consists of a variety of non starch polysaccharides, resistant to hydrolysis by human alimentary enzymes, which include all indigestible polysaccharides (pectins, soluble and insoluble hemicelluloses, cellulose, β -glucans, gums) and lignin (Prosky, 1999; Lamghari et al., 2000; Gallaher and Schneeman, 2001).

The results of the dietary fibre analysis are summarized in Table 2 (from enzymatic gravimetric method) and Table 3 (from non-enzymatic gravimetric method).

Table 2 shown the insoluble dietary fibre (IDF = 75 g/100 g), soluble dietary fibre (SDF = 15 g/100 g) and total dietary fibre (TDF(1) = IDF + SDF = 90 g/100 g) content and the ratio between IDF and SDF (5:1). Fiber is often classified as soluble dietary fibre (SDF) and insoluble dietary fibre (IDF). Because it was thought that this categorization might provide a simple way to predict physiological and physicochemical functions. Fibers with 15% of SDF are able to bind and retain several times their weight of water (Gorinstein et al., 2001, Figuerola, 2004).

Also in Table 2, it can be observed that the TDF(2) amount (96 g/100 g), determined by a separate analysis (as described in section Determination of TDF), was close to the proportions (90 g/100 g) obtained by summing IDF and SDF. In general, natural product with more than 60% of TDF (dry matter basis); could be considered as rich source of dietary fibre, suitable for use as food ingredient (important for both, dietary and functional properties) (Femenia et al., 1997; Larrauri, 1999; Jaime et al., 2002; Schneeman, 1987).

Table2: Dietary fibre composition(g/100g on DM) by enzymatic gravimetric method.

	TDF(1) (IDF + SDF): Pectin, Soluble and Insoluble Hemicelluloses, Cellulose, Lignin	Composition of TDF(1)			TDF (2): Pectin, Soluble and Insoluble Hemicelluloses, Cellulose, Lignin
		IDF: Insoluble Hemicelluloses, Cellulose, Lignin	SDF: Pectin, Soluble Hemicelluloses	IDF/SDF ratio	
Carob grain tegument	90 ± 2.41	75 ± 3.20	15 ± 1.63	5:1	96 ± 1.24

IDF = Insoluble Dietary Fiber; SDF = Soluble Dietary Fiber; TDF = Total Dietary Fiber (TDF (1) determined by summing the IDF and SDF; TDF (2) determined independently)

Table 3 shows the percentage of each components of the total crude fibre (determined as Neutral Dietary Fiber (NDF)) in carob seed hull. Insoluble hemicelluloses, cellulose and lignin fractions accounted for 20 g/100 g, 33 g/100 g and 9 g/100 g, respectively, on dry weight basis.

Table 3: Crude dietary fibre composition (g/100g on DM) by chemical-gravimetric method

	NDF: insoluble Hemicelluloses, Cellulose, Lignin	Composition of NDF		
		Insoluble Hemicelluloses	Cellulose	Lignin
Carob grain husk	62 ± 3.19	20 ± 2.22	33 ± 2.35	9 ± 1.23

NDF = Neutral Dietary Fiber

Theoretically, NDF composition is similar to the IDF composition; although appears evident difference in their content values (62% NDF and 75% IDF). This difference confirms that dietary fibre content is over-estimated by Enzymatic-Gravimetric Method and under-estimated by Chemical-Gravimetric Method.

In general, the results obtained suggest that carob seed hull was source of abundant dietary fibre for locust bean gum (LBG) enrichment.

There are a number of published reports indicate composition and health benefits associated with an increased intake of dietary fibre (including pectins, hemicelluloses, cellulose and lignin, as well as gums). These publications report that:

Published by European Centre for Research Training and Development UK (www.eajournals.org)

Pectins are the polysaccharides found in plant cell walls as well as in the outer skin and rind of fruits and vegetables, e.g. the rind of an orange contains 30% pectin, an apple peel 15%, and onion skin 12%. Pectins are mainly composed of chains of galacturonic acid interspersed with units of rhamnose and are branched with chains of pentose and hexose units (Ridley et al., 2009). They are soluble in hot water and then form gels on cooling hence used as gelling and thickening agents in various food products. Cholesterol lowering effects of pectin is due to its gel-forming capacity (Slavin et al., 1987). Pectin lowers cholesterol by binding the cholesterol and bile acids in the gut and promoting their excretion.

Hemicelluloses (soluble and insoluble) are also a component polysaccharide of plant cell wall (Ferguson, 2001). It differs from cellulose in having monomer units other than glucose. Hemicellulose includes both linear and branched molecules, smaller than cellulose, typically containing 50–200 pentose units (xylose and arabinose) and hexose units (glucose, galactose, mannose, rhamnose, glucuronic and galacturonic acids). The name hemicellulose therefore describes a heterogeneous group of chemical structures that are present in plant foods in water soluble and insoluble forms (Hu et al., 2009). About one third of the dietary fiber in vegetables, fruits, legumes and nuts consists of hemicelluloses. Hemicelluloses promote regular bowel movements by increasing hydration of the stool. Hemicelluloses also directly bind cholesterol in the gut, preventing cholesterol absorption (Mudgil et al., 2012). Bacteria in the gut digest hemicelluloses increasing the number of beneficial bacteria in the gut and creating short-chain fatty acids which colon cells use as fuel and decrease cholesterol. This activity of dietary fiber is known as prebiotic activity. Prebiotic substances are the indigestible food component that beneficially influences the host organism by selective stimulation of growth and activity of beneficial bacteria such as Lactobacilli and Bifidobacteria in the colon, and thus improves the host health.

Cellulose is the most abundant polysaccharide found in nature. It is a major component of the cell wall of most plants and hence, present in fruits, vegetables and cereals. It is a linear and unbranched polysaccharide consisting of up to 10,000 glucose monomer units per molecule. The linear molecules are packed closely together as long fiber and are very insoluble and resistant to digestion by human enzymes. Cellulose forms about one fourth of the dietary fiber in grains and fruit and one third in vegetables and nuts. Wheat bran is rich source of cellulose or insoluble fiber (Ferguson, 2001). Being insoluble in water it has an ability to bind water which helps in increasing fecal volume and thus promoting regular bowel movements. Although humans are not able to digest cellulose but its partial digestion occurs in the gut by beneficial microflora. About 50% of cellulose is degraded by natural fermentation in colon and produce significant amount of short-chain fatty acids which feed our intestinal cells.

Lignin is a non-digestible compound made of phenols (aromatic alcohols) and other molecules. It is not a carbohydrate, but is considered an insoluble fiber. It is found in the cell walls of vascular plants and in seeds. Lignin is not digested in the small bowel and is poorly fermented by normal colonic bacteria. It is insoluble in water, but it absorbs water and thus gives bulk to the stool and it might help to prevent diverticulosis and the growth of cancer cells (Bitsch, 1979).

Gums such as *Locust bean gum* and guar gum from seeds endosperm are composed of reserve polysaccharides (hemicelluloses) and are included in the hydrocolloids. The hydrocolloids

Published by European Centre for Research Training and Development UK (www.eajournals.org)

comprise a wide range of mixed viscous polysaccharides. These hydrocolloids are used in small amounts as gelling, thickening, stabilizing and emulsifying agents in certain food products (Saha et al., 2010). Gums and other mucilages, including psyllium seed husk and konjac root glucomannan, are perhaps the most potent cholesterol-lowering agents of the gel-forming fibers (Slavin et Greenberg, 2003). In addition, These hydrocolloids have been shown to reduce fasting and after-meal glucose and insulin levels in both healthy and diabetic subjects; and decreased body weight and hunger ratings when taken with meals by obese subjects (Mudgil et al., 2014).

In general, health benefits associated with dietary fibre itself or a diet rich in dietary fibre include the prevention, reduction and treatment of some diseases, such as constipation and colonic-rectal cancer (increases fecal bulk, stimulates colonic fermentation), coronary heart diseases (reduces pre-prandial cholesterol and lipid levels), diabetes (reduces postprandial blood glucose and reduces insulin responses), diverticulitis, appendicitis, varicose veins, obesity and gallstones (Anderson et al., 1994; Gorinstein et al., 2001).

Dietary fibre can also impart some functional properties to foods, e.g. increase water holding capacity, oil holding capacity, emulsification and/or gel formation. Dietary fibre incorporated into various food products (bread, pasta, ice cream, yoghurt, minced meat product, bakery products, dairy, jams, soups) can substantially modify textural properties, avoid syneresis (the separation of liquid from a gel caused by contraction), stabilise high fat food and emulsions, and improve shelf-life (Femenia et al., 1997; Gallaher and Schneeman, 2001; Elleuch et al., 2010; Mudgil et al., 2013)

We hope our data and interpretation stimulate some further work over some functional properties of carob seed hull fibres, such as water-holding capacity (WHC), oil-holding capacity (OHC), swelling capacity (SWC), viscosity or gel formation, bile acid binding capacity, and cation-exchange capacity could be undertaken for understand the physiological effect of the caro seed tegument dietary fibre.

CONCLUSION

The dietary fibres analysis shown that carob seed coat contains about 90% TDF (75% IDF + 15% SDF) and 62% NDF according to the method used (enzymatic or non-enzymatic method). The NDF composition revealed the presence of 20% of insoluble hemicelluloses, 33% of cellulose and 9% of lignin fractions. This result suggests that this product could be considered as rich source of dietary fibre, suitable for use as food ingredient.

We can concluded, in view of all results obtained, that the “accidental incorporation” (contamination) of these small husk fractions, during gum primary extraction process, may be regarded, as a potential fibre source for Locust bean gum flour enrichment.

Acknowledgements

We thank Jean Rémont from T.A.S.A. (Benjaraf, Malaga, Spain) for the supply of raw

Published by European Centre for Research Training and Development UK (www.eajournals.org)
materials (carob seeds) and Ir Roiseux and Pr Blecker (SAF-ULg-GxABT, Belgium) for their assistance in fibers measurements.

REFERENCES

- Anderson, J.W., Smith, B.M. and Guftanson, N.S., 1994. Health benefit and practical aspects of high-fibre diets. *American Journal of Clinical Nutrition* 595, pp. 1242–1247.
- AOAC. (1984). *Official Methods of Analysis* (14th ed.). Arlington, VA: AOAC International.
- Battle, I., & Tous, J. (1997). Properties. Agronomy: Processing. In *Carob tree, Ceratonia siliqua* L. IPGRI.CGIAR. Rome. Italy. Pp 24-29, 61-62.
- Battle, I., & Tous, J. (1997). Properties. Agronomy: Processing. In *Carob tree, Ceratonia siliqua* L. IPGRI.CGIAR. Rome. Italy. Pp 24-29, 61-62.
- Bitsch R. Leber Magen Darm. 1979. Bulk vegetable material in human nutrition. *Leber Magen Darm.* Dec;9(6):300-6. PubMed
- Bouzouita, N., Khaldi, A., Zgoulli, S., Chebil, L., Chekki, R., Chaabouni, M.M. and Thonart, P., (2007). “The Analysis of Crude and Purified Locust Bean Gum: A Comparison of Samples from Different Carob Tree Populations in Tunisia”, *Food Chemistry*, vol.101, pp.1508-1515.
- da Silva, J.A.L., Gonçalves, M.P., , 1990. “Studies on a purification method for locust bean gum by precipitation with isopropanol”, *Food Hydrocolloids*, vol.4, pp.277–287.
- Dakia, P.A., Wathélet, B. & Paquot, M. 2007. Isolation and chemical evaluation of the carob (*Ceratonia siliqua* L) seeds germ. *Food Chemistry*. 102(4), 1368-1374.
- Dakia, P.A., Christophe Blecker, Christelle Robert, Wathélet, B., Paquot, M., 2008. Composition and physicochemical properties of locust bean gum extracted from whole seeds by acid or water dehulling pre-treatment. *Food Hydrocolloids*, vol.22, no.5, pp.807-818.
- Dakia, P.A., Wathélet, B., Paquot M., , 2010, Influence de la teneur en galactose sur les interactions moléculaires et sur les propriétés physico-chimiques des galactomannanes en solution. *Biotechnol. Agron. Soc. Environ.* Vol.14, no.1, pp.213-223.
- Dakia, P. A., 2011. Carob (*Ceratonia siliqua* L.): Seeds, endosperm and germ composition, and application to health. In V. R. Preedy, R. R. Watson, V. B. Patel (Editors), *Nuts & Seeds in Health and Disease Prevention* (1st ed.) (pp 293-299). London, Burlington, San Diego: Academic Press is an imprint of Elsevier.
- Dakia, P.A., Combo A. M-M., Yapo M. B., Paquot M. (2017a), Effect of the Seed Morphology on the Separation Yield, Chemical Characteristics and Thickening Capacity of Carob (*Ceratonia siliqua* L.) Gums, *Asian Journal of Agriculture and Food Sciences*, vol 05, No 05, pp 216-224
- Dakia, P.A., Combo A. M-M., Yapo M. B., Brou K.D., Paquot M. (2017b), Carob (*Ceratonia siliqua* L) Seed Tegument Separation and Analysis: Comparison with Locust Bean Gum Hot-Water-Insoluble Residue Monosaccharide’s Composition. *International Journal of New Technology and Research*, 3 (11), 30-35.
- Dakia, P.A., Combo A. M. M., Gbogouri G. A., Paquot M. (2018), Physicochemical Characteristics Of Locust Bean Gum Purified Fractions Obtained By Temperature Fractionation. *European Journal of Food Science and Technology*, 6 (4), 11-27.

Published by European Centre for Research Training and Development UK (www.eajournals.org)

- Dea, I.C.M., & Morrison, A., 1975. Chemistry and interactions of seed galactomannans. *Advances in Carbohydrate Chemistry and Biochemistry*, 31, 241–242.
- El Batal H., Hasib, A. Ouatmane, A. Boulli, A. Dehbi, F. Jaouad A., , 2013. “Yield and composition of carob bean gum produced from different Moroccan populations of carob (*Ceratonia siliqua* L.)”, *Journal of Materials and Environmental Science*. Vol.4, no.2, pp.309-314.
- Elleuch M., Bedigian D., Roiseux O., Besbes S., Blecker C., Attia H., 2011. Dietary fibre and fibre-rich by-products of food processing: Characterisation, technological, functionality and commercial applications: A review. *Food Chemistry*, 124, pp. 411-421
- Englyst, H.N. and Cumming, J.H., 1984. Simplified method for the measurement of total non-starch polysaccharides by gas-chromatography of constituents sugars as alditol acetates. *Analyst*, 109, 937–942.
- Ensminger, Ensminger, Konlande, & Robson, 1994. Carob *Ceratonia siliqua*. In *Food and Nutrition Encyclopedia*. by CRC Press, Inc, USA, 2nd Ed. Vol. 1. pp 346-348.
- Femenia, A., Lefebvre, C., Thebaudin, Y., Robertson, J. and Bourgeois, C., 1997. Physical and sensory properties of model foods supplemented with cauliflower fiber. *Journal of Food Science*, 62(4), 635–639.
- Ferguson L.R., Chavan R.R., Harris P.J., 2001. Changing concepts of dietary fiber: implications for carcinogenesis. *Nutrition and Cancer*, 30, 155–169.
- Figuerola F., Hurtado M.L., Estévez A.M., Chiffelle I., Asenjo G., 2005. Fibre concentrates from apple pomace and citrus peel as potential source for food enrichment, *Food Chemistry*, 91, pp. 395-401
- Folch, Lees, M., & Stanley, S.G.H., 1957. A simple method for the isolation and purification of total lipids from animal tissues. *Journal of Biological Chemistry*, 226, 497–509.
- Gallaher, D., & Schneeman, B.O. (2001). Dietary fiber. In: B. Bowman & R. Russel (Eds.). *Present knowledge in nutrition* (8th ed., 805p). Washington, DC: ILSI
- Gillet S., Blecker, C., Aguedo, M., Laurent, P., Paquot, M., Richel A., 2014. Impact of purification and process on the chemical structure and physical properties of locust bean gum, *Carbohydrate Polymer*. Vol.108, pp.159–168,.
- Gillet, S., Aguedo, M., Petrut, R, Olive, G., Anastas, P., Blecker, C., Richel, A., 2017. “Structure impact of two galactomannan fractions on their viscosity properties in dilute solution, unperturbed state and gel state”. *International Journal of Biological Macromolecules*, vol.96, pp.550–559.
- Gorinstein, S., Zachwieja, Z., Folta, M., Barton, H., Piotrowicz, J., Zember, M., Weisz, M., Trakhtenberg, S. and Martín-Belloso, O., 2001. Comparative content of dietary fiber, total phenolics, and minerals in persimmons and apples. *Journal of Agricultural and Food Chemistry* 49, pp. 952–957.
- Haddarah A., Ismail A., Bassal A., Hamieh T., Ghoul M., 2013. “Morphological and chemical variability of lebanese carob varieties”. *European scientific journal*, vol.9, no.18, pp.353–369.
- Haddarah, A., Bassal, A., Ismail, A., Gaiani, C., Ioannou, I., Charbonnel, C., Hamieh, T., Ghoul M., 2014. “The structural characteristics and rheological properties of Lebanese locust bean gum.” *Journal of Food Engineering*, vol.120, pp.204–214.

Published by European Centre for Research Training and Development UK (www.eajournals.org)

- Hariri, A., Ouis, N., Sahnouni, F., Bouhadi, D., 2009. Mise en oeuvre de la fermentation de certains ferments lactiques dans des milieux a base des extraits de caroube. *Rev. microbiol. ind. san et environn.*, 18, 37-55.
- Haralampu, S.G., 2000. Resistant starch—a review of the physical properties and biological impact of RS₃. *Carbohydrate Polymers*41, pp. 285–292
- Hefty (1953). U.K. Patent N°14813/53.
- Hu, G., Huang, S., Cao, S., and Ma, Z., 2009. Effect of enrichment with hemicellulose from rice bran on chemical and functional properties of bread. *Food Chemistry*, 115: 839–842.
- Jaime, L., Mollá, E., Fernández, A., Martín-Cabrejas, M., López Andreu, F. and Esteban, R., 2002. Structural carbohydrates differences and potential source of dietary fiber of onion (*Allium cepa* L.) tissues. *Journal of Agricultural and Food Chemistry* 50, pp. 122–128
- Kıvrak N. E., Askın B., Kucukoner E, 2015 “Comparison of Some Physicochemical Properties of Locust Bean Seeds Gum Extracted by Acid and Water Pre-Treatments”, *Food and Nutrition Sciences*, vol.6, pp.278-286,.
- Lamghari, R., Sánchez, C., El Boustani, E., Maucourt, N.M., Sauvaire, Y., Mejean, L. and Villaume, C., (2000). Comparison of effects of prickly pear (*Opuntia ficus indica* sp.) fruits, arabic gum and citrus pectin on viscosity and in vitro digestibility of casein. *Journal of the Science of Food and Agriculture*80, 359–364.
- Larrauri, J.A., 1999. New approaches in the preparation of high dietary fibre powders from fruits by-products. *Trends in Food Science and Technology*, 10, pp. 3–8.
- Lee S.C., Prosky L. and DeVries J.W., 1992. Determination of total, soluble, and insoluble dietary fibre in foods—enzymatic—gravimetric method, MES—TRIS buffer: collaborative study. *Journal of AOAC International*75, pp. 395–416.
- McCleary B. V. and Matheson N. K. (1975). Galactomannan structure and β -mannanase and β -mannosidase activity in germinating legume seeds. *Phytochemistry*, 14(5-6), 1187-1194
- Mudgil, D., Barak S. and Khatkar B.S.. 2012. X-ray diffraction, IR spectroscopy and thermal characterization of partially hydrolyzed guar gum. *International Journal of Biological Macromolecules*. 50:1035-1039.
- Mudgil D., Barak S.. 2013. Composition, properties and health benefits of indigestible carbohydrate polymers as dietary fiber: A review. *International Journal of Biological Macromolecules* 61, 1– 6
- Mudgil D., Barak S., Khatkar B.S., 2014. Guar gum: processing, properties and food applications-A Review. *Journal of Food Science and Technology*, 2014 Mar; 51(3),409-18.
- Neukom, H. (1988). Carob bean gum: properties and applications. in Fito P. & Mulet A., (Eds.), *Proceedings of the II International Carob Symposium* (Pp.551-555). Valencia, Spain.
- Prosky Leon (1999) What is fibre? Current controversies. *Trends in Food Science & Technology*, 10(8), 271-275
- Prosky Leon (2000). What is fiber? *journal of aoac international*, vol. 83, no. 4
- Prosky L. and Mugford D. (2001). Letters to the Editors: Resistant starch and dietary fiber. *Carbohydrate Polymers*, 44(1), 81
- Saha D. and Bhattacharya S., 2010, “Hydrocolloids as Thickening and Gelling Agents in

Published by European Centre for Research Training and Development UK (www.eajournals.org)

Food: A Critical Review,” *Journal of Food Science and Technology*, Vol. 47, No. 6, pp. 587-597

- Sandolo, C., Coviello, T., Matricardi, P., Alhaique, F., 2007. Characterization of polysaccharide hydrogels for modified drug delivery. *Eur. Biophys. J.* 36 (7), 693–700.
- Schneeman, B.O., 1987. Soluble vs insoluble fiber – different physiological responses. *Food Technology* 47 2, pp. 81–82.
- Sigge G.O., lipumbu L., Britz T.J. (2011) Proximate composition of carob cultivars growing in South Africa, *South African Journal of Plant and Soil*, 28:1, 17-22
- Slavin, J.L., 1987. Dietary Fiber Classification, Chemical Analyses, and Food Sources. *Journal of the American Dietetic Association*, 87, 1164-1171
- Slavin, J.L. and Greenberg, N.A., 2003. Partially hydrolyzed guar gum clinical *nutrition* uses. *Nutrition*, 19: 549-552.
- Ridley, B.L., O'Neill, M.A. and Mohnen, D., 2001. Pectins: Structure, Biosynthesis, and Oligogalacturonide-Related Signaling. *Phytochemistry*, 57, 929-967.
- Van Soest P.J.; Wine R H. (1967). Use of detergents in the analysis of fibrous feeds. IV. Determination of plant cell-wall constituents. *J. Assn. Offic. Anal. Chem.* 50:50-5