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Characterization and Functional Properties of an Oat Gum Extracted from a Drought Harvested Oat (*Avena sativa*)

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Abstract An oat gum was extracted from whole seeds of a drought harvested oat (*Avena sativa*). Oat gum presented a β-glucan content of 65%(w/w) and an intrinsic viscosity of 141 mL/g. Gelling capability of oat gum at different concentrations was investigated. Gel hardness increased from 0.08 to 0.25 N as the oat gum concentration changed from 5 to 10%(w/v). Whippability, foam stability, emulsion stability, and reduced viscosity of oat gum at different pH were also investigated. Oat gum whippability was maximum at pH 7 (146%), while the higher foam and emulsion stability values were found at pH 9 (88 and 96%, respectively). The gum reduced viscosity increased from 715 to 958 mL/g as the pH changed from 7 to 9. Oat gum shows great potential as a gel forming, thickening, and stabilizing agent.

Keywords: oat gum, β-glucan, gelation, emulsion, functional property, food industry

Introduction

The main component of soluble dietary fibre of oat is (1→3), (1→4)-β-D-glucan, also referred to as β-glucan (1). β-Glucan is a linear polysaccharide made up entirely of sequences of (1→4)-linked D-glucopyranosyl units separated by single (1→3)-β-linked units (2). β-Glucan is a cell wall polysaccharide found in the endosperm and in the subaleurone layer of oat and barley (3). Oat (*Avena sativa)* contains from 3.2 to 6.8% of β-glucan (1), which varies with cultivar and environmental effects (3).

Research on oat (*A. sativa)* has been intensified in the last years as in clinical studies oat β-glucan was shown to reduce serum cholesterol levels and attenuate postprandial blood glucose and insulin responses in a viscosity related fashion (4,5). On the other hand, because of the high viscosity of their solutions, β-glucan had a high potential application as food texturizer (6). In addition, some β-glucan can form physical gels which have been proposed as fat mimetic and as encapsulation agent (7).

Oat is extensively planted as a forage crop in northern Mexico, where rainfall has an erratic distribution and therefore, seeds yields are low. In fact, oat cultivars as 'Cuauhtemoc' have been developed for drought conditions. As one of important animal feed resources, mexican oat cultivars have been studied on the basis of forage yield and nutritional value. Nevertheless, to our knowledge, studies on the characterization and functional properties of β-glucan from oat Mexican grains harvested under drought conditions, has not been reported elsewhere. Therefore, the aim of this study was to extract a β-glucan-enriched oat gum from whole seeds of drought harvested oat cultivar 'Cuauhtemoc' and to investigate its composition and functional properties.

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Materials and Methods

Materials Whole oat seeds from oat (*Avena sativa*) cultivar 'Cuauhtemoc' harvested under drought conditions were provided by the National Institute for Investigation in Forestry, Agriculture, and Animal Production in Mexico (INIFAP). Whole oat seeds were milled down to 0.84 mm particle size using a M20 Universal Mill (IKA®, Werke Staufen, Germany). Oat gum was water extracted from milled seeds (1 kg/3 L) for 15 min at 25°C. The water extract was then centrifugated (12,096×g, 20°C, 15 min) and supernatant recovered. Supernatant was precipitated in 65% ethanol treated for 4 hr at 4°C. Precipitate was recovered and dried by solvent exchange (80%, v/v ethanol, absolute ethanol, and acetone) to give oat gum (8). All chemicals were analytical grade purchased from Sigma-Aldrich (St. Louis, MO, USA).

Chemical analysis Fat, fibre, protein, and ash content in whole oat seeds were performed according to the AOAC approved methods (9). Starch content in whole oat seeds and oat gum was determined using a Megazyme assay kit (Wicklow, Ireland) (10). The β-glucan content in the oat gum and in the whole oat seeds was determined according to a method previously reported (11). The method involves dispersing the samples in a phosphate buffer (4.0 mL, 20 mM, pH 6.5) and incubating with lichenase (EC 3.2.1.73), at 50°C for 60 min. A second step with β-glucosidase (EC

3.2.1.21) at 50°C for 20 min hydrolyses the oligosaccharides to glucose. Glucose is then converted to a colored substance and analyzed spectrophotometrically at 510 nm with a Cary 1E Varian spectrometer (Varian, St. Helens, Australia). Neutral sugars content in oat gum was determined after hydrolysis of the gum with 2 N trifluoroacetic acid at 120°C for 2 hr. The evaporated extract was solubilized in 500 μL of water. Samples were filtered through 0.45-μm (Whatman) and analyzed by high performance liquid chromatography (HPLC) using a Supelcogel Pb column (300×7.8 mm; Supelco, Inc., Bellefont, PA, USA) eluted with water (filtered 0.2-μm, Whatman) at 0.6 mL/min and 80°C. Inositol was used as internal standard. A refractive index detector Star 9040 (Varian) was used (12). Ferulic acid content was determined in oat gum and whole oat seeds after saponification with 2 N NaOH for 2 hr in the dark at 35°C under argon by HPLC. Ferulic acid was extracted twice with diethyl ether, and evaporated at 30°C under argon. The dried extracts was solubilized in 0.50 mL methanol/water/acetic acid (40/59/01), filtered (0.45 μm), and injected (20 μL) into HPLC using a Supelcosil LC-18-DB (250×4.6 mm) (Supelco, Inc.) column. Elution was performed using methanol/water/acetic acid (40/59/01) at 0.6 mL/min at 35°C. 3,4,5-Trimethoxy-trans-cinnamic acid (TMCA) was used as internal standard. Detection was by UV absorbance at 280 nm. A Varian 9012 photodiode array detector (Varian) was used (13). Protein content in oat gum was determined according to Bradford (14). Ash content was determined according to the AACC Approved Method (15). Results were reported on a dry weight basis (d.b.).

Gelation Oat gum solutions (5, 8, and 10%, w/v) were prepared by gentle stirring samples in double distilled water at 75°C until complete solubilization of the material. Gels were allowed to form for 2 hr at 25°C (16).

Gel hardness The hardness of 5, 8, and 10%(w/v) oat gum gels, freshly made (2 hr) in 6-mL glass flasks of 30-mm height and 25-mm internal diameter was analyzed with a TA.XT2i Texture Analyzer (RHEO Stable Micro Systems, Haslemere, England). The gels were deformed by compression at a constant speed of 1.0 mm/sec to a distance of 4 mm from the gel surface using a cylindrical plunger (diameter 15-mm). The peak height at 4 mm compression was called gel hardness (17).

Whippability and foam stability Three oat gum solutions were prepared by adding 2.5 g gum in 100 mL water and the pH was adjusted to 7, 8, or 9 with 4 N NaOH. Oat gum solutions were whipped for 2 min using a hand-held food mixer high speed (350 rpm) Mini MP170 (Robot-Coupe, Jackson, MI, USA) and measuring volumes before and after whipping. Whippability was reported as the percentage increase in volume due to whipping. Foams were slowly transferred to a 1,000-mL graduated cylinder and allowed to set at room temperature (21°C) for 2 hr. Volume of foam after 2 hr as a percentage of original volume was reported as foam stability (18).

Emulsion stability Emulsion stabilizing capacity was determined as reported before (18). Oat gum (0.02 g) was dispersed in 40 mL water at pH 7, 8, and 9. Parafin oil (60 mL) was added slowly and the mixture was blended for 2 min using a Brinkmann polytron homogenizer PT3000 (Westbury, NY, USA) at 17,400 rpm and 21°C. Aliquots of emulsion were transfered into 50 mL graduated tubes and centrifugated for 15 min at 2,700×g. Volumes of separated phases were recorded. Emulsion stability was reported as the volume of emulsion remaining unseparated after centrifugation as a percentage of original volume.

Viscosity Specific viscosity of oat gum solutions at 0.25%(w/v) in water at pH 7, 8, and 9 was measured with an AVS 400 capillary viscosimeter (Schott Geräte, Hofheim, Germany), equipped with an Oswald capillary tube (flow water time 75.15 sec). The specific viscosity was related to the oat gum concentration to obtain their reduced viscosity (mL/g). The intrinsic viscosity of oat gum was determined as reported before (17).

Statistical analysis The experimental data were analyzed with the Statistical Analysis System software (SAS Institute, Cary, NC, USA). The significance of difference was calculated using Tuckey's test ($p \leq 0.05$).

Results and Discussion

Chemical composition of oat seeds The chemical composition of whole oat seeds is presented in Table 1. Fat (9.6%), fibre (2.9%), protein (13.2%), ash (2.3%), and starch (63%) contents were in the range of values reported for oat genotypes grown in different environments (19-21). The β-glucan content in 'Cuauhtemoc' variety was 3.9% (d.b.), which is in the range of that reported for other oat varieties (3.7-5.0%, w/w) (22). The ferulic acid content in 'Cuauhtemoc' oat variety was higher than that reported (22) in other oat cultivars from Poland (0.011-0.008% d.b.).

Oat gum extraction and characterization Oat gum yield was 2.3%(w oat gum/w oat seeds), which means that 60% of the β-glucan initially present in the oat seed (3.9%, w β-glucan/w oat seed d.b.) was recovered. Beer *et al.* (23) reported that 60-65% of total β-glucan can be extracted from oat seeds by water extraction at 90°C. Similar conditions were used in our extraction procedure, explainning the similar yield value. Chemical composition of oat gum is presented in Table 2. The extraction procedure adopted in the present study provided an oat gum with β-glucan as the major component (65% d.b.). Starch (15.6%),

Table 1. Composition of whole oat seeds cultivar 'Cuauhtémoc'

Fat	9.60±0.20[1)]
Fibre	2.90±0.20
Protein	13.20±0.14
β-Glucan	3.90±0.02
Ash	2.30±0.10
Ferulic acid	0.070±0.001
Starch	68.00±0.00

[1)]Results are expressed in g/100 g dry matter; All results are obtained from triplicates.

Table 2. Composition of oat gum

β-Glucan	65.00±1.50[1)]
Starch	15.64±0.90
Arabinose	9.00±0.80
Xylose	4.00±1.10
Protein	3.64±0.10
Ash	2.70±0.30
Ferulic acid	0.020±0.001

[1)]Results are expressed in g/100 g oat gum matter; All results are obtained from triplicates.

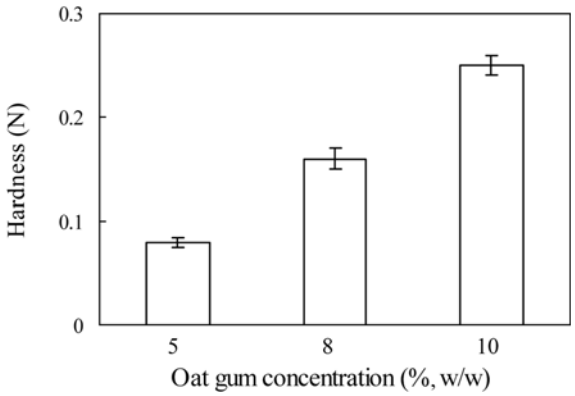

Fig. 1. Hardness of oat gum gels at different concentrations. Values are means of triplicates measurements.

arabinose (9%), xylose (4%), protein (3.6%), ash (2.7%), and ferulic acid (0.02%) were also detected in this oat gum. Coextraction of starch (0.8-1.7%) has been reported when extracting a β-glucan enriched barley gum under similar conditions but a lower starch content was reported in barley flour (55%, w starch/w barley flour) (18). The ferulic acid content in the whole oat seeds (0.07% d.b.) was higher to that found in the oat gum (0.02% d.b.), which could indicate that most of the ferulic acid residues in 'Cuauhtemoc' oat seeds are mainly attached to other chemical structures such as arabinoxylans. The intrinsic viscosity value of oat gum was 141 mL/g and the viscosimetric molecular weight was 50 kDa which are lower to that reported for other oat β-glucan enriched gums (24).

Oat gum functional properties Solubilization of oat gum in hot water produced oat gum solutions at 5, 8, and 10%(w/v) which formed firm and white gels after 2 hr at 25°C. The hardness of oat gum gels at different concentrations is reported in Fig. 1. A 68% increase in the gel hardness was recorded by increasing the oat gum concentration from 5 to 10%(w/v). This result could be related to the polysaccharide chain aggregation phenomena, which is promoted as the polysaccharide concentration increase. As a fact, β-glucan gels belong to the category of physically cross-linked gels whose 3-dimensional structure is stabilized mainly by multiple inter and intra chain hydrogen bonds in the junction zones of the polymeric network (1). From a functional point of view, it has been reported that the gel formation properties of β-glucan increase intestinal transit time (25). The gel network that is formed could also slow down the digestion by retarding diffusion and diminishing contact between gastrointestinal enzymes and their substrates (16).

Table 3. Functional properties of oat gum

pH	Whippability (%)	Foam stability (%)	Emulsion stability (%)	Reduced viscosity (mL/g)
7	146[a1)]	75[b]	74[c]	715[c]
8	142[a]	80[b]	90[b]	854[b]
9	116[b]	88[a]	96[a]	958[a]

[1)]Means in a column with different letters are significantly different ($p \leq 0.05$); All results are obtained from triplicates.

Whippability, foam and emulsion stability, and reduced viscosity of oat gum dispersions at different pH values are presented in Table 3. Whippability significantly decreased ($p \leq 0.05$) from 146 to 116% as the pH increased from 7 to 9. Coextracted proteins in the oat gum may be expected to make a higher contribution to whippability at pH 7 than at pH 9. Nevertheless, foam and emulsion stability increased ($p \leq 0.05$) from 75 to 88% and from 74 to 96%, respectively, when the pH augmented from 7 to 9. This increase in foam and emulsion stability could be related to the fact that oat gum reduced viscosity increased ($p \leq 0.05$) from 715 to 958 mL/g as the pH changed from 7 to 9. It is known that polysaccharides contributed to the stability of foam and emulsion systems mainly by increasing the viscosity of the aqueous phase. They do not interact with the hydrophobic phase since they are not true surfactants. Coalescence of air bubbles and oil droplets in foam and emulsion systems, respectively, is hindered by a viscous aqueous phase. Therefore, the increase in reduced viscosity of oat gum solutions as the pH augmented from 7 to 9 could explain the higher foam and emulsion stability values at higher pH values.

An oat gum with β-glucan as major component can be recovered from a drought harvested oat cultivar. The oat gum recovered formed physical gels. An increase in gum concentration from 5 to 10%(w/v) highly improved the gels hardness. There was a significant effect ($p \leq 0.05$) of pH on oat gum functionality. Maximum foam and emulsion stability and reduced viscosity were achieved at pH 9. Oat β-glucan gum presented great potential as encapsulation agent, thickener or stabilizer fort the food industry. Recuperation of this oat gum from a low value oat cultivar could represent a commercial advantage face to other gums commonly used in the food industry.

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