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Structural Characterization of Corn Fiber Gums from Coarse and Fine Fiber and a Study of Their Emulsifying Properties

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The stabilities of orange oil emulsions stabilized with various concentrations of two different types of corn fiber gum (CFG-1 and 2) isolated from coarse (pericarp) and fine (endosperm) fiber from corn wet milling have been studied. The emulsion stabilities in all these studies increased with increasing gum concentration up to a gum-to-oil ratio of 0.05, and after that it either levels off or changes very slightly. These results indicate that only 0.25% of CFG is required to make stable emulsion containing 5% orange oil under the experimental conditions used in this study. At this CFG concentration, CFG-2 from each fiber source was found to be a superior emulsifier relative to the corresponding CFG-1 from each source in a 10-day emulsion stability study at room temperature. The emulsion stability was also investigated by confocal laser scanning microscopy measurement, and it was found that CFG-1 and 2 from both coarse and fine fiber made stable emulsions with an average particle size of less than 1 μm for 10 days at room temperature. Sugar composition analysis of CFGs from both sources indicated that they were typical galactoglucuronoarabinoxylans containing mainly 55−59% xylose, 29−36% arabinose, and 4−6% galactose as neutral sugars and 3−5% glucuronic acid. Methylation analysis revealed a highly branched structure of all CFGs, in which only 16−25% of the 1→ 4-linked xylose residues were not substituted at O-2 and/or O-3. Arabinose is present both as a terminal residue and at branch points.

KEYWORDS: Corn fiber; arabinoxylan; emulsion stability; emulsifier; oil-in-water emulsion; homogenization

INTRODUCTION

Corn fiber gum (CFG), a potential emulsifier for oil-in-water emulsion systems (*1*, *2*) can be isolated from a low-valued byproduct (corn fiber) of corn milling industries. Wet milling of corn kernels produces two kinds of fibrous material called coarse and fine fiber. The coarse fiber originates from the outer pericarp layer and tip cap, and the fine fiber originates from the endosperm portion of the corn kernel. CFGs isolated from these two fiber sources have similar sugar composition (*3*), but they differ in their protein, lipid, and phenolic acid contents (*4*). Previously, we reported the emulsifying properties of CFG isolated from different fiber sources (*1*, *2*), but the present investigation is undertaken for identifying the emulsifying properties of CFG isolated separately from coarse and fine corn fiber. Corn fiber, a byproduct of the commercial corn wet milling process is generally used in animal feeds with gluten (corn protein). Over 4 million tons of corn fiber are produced annually in the United States, but other than its use in corn gluten feed, a low-valued feed ingredient, there have been very few successful commercial uses (*5*, *6*). The value-added product, CFG (also called hemicellulose B or Hemi. B) may have many potential uses in the food and non-food industries and could represent a new use for corn fiber.

Oil-in-water emulsions are prepared by homogenizing oil and water together in the presence of an emulsifier (*7*). An emulsifier is a surface-active molecule, which adsorbs to the surface of oil droplets and forms stiff resistant viscoelastic protective layers. The formed protective layers prevent the oil droplets from fusing together (flocculating and/or coalescing) due to steric hindrance and/or repulsion. An emulsifier also helps to reduce oil−water interfacial tension, making the disruption of emulsion droplets easier during the emulsification process (*8*, *9*). Although substantial work has been done and several methods have been developed for the determination of emulsion properties (*10*, *11*), the photometric method of Pearce and Kinsella (*12*) has become very popular for routine analysis. In comparison to other methods to study emulsions, in which droplet size in the dispersed phase is measured by microscopy or different particle size analysis, this method is more indirect, but is far simpler. This method is based on turbidity measurement of the dispersed particles in the emulsion, which correlates directly with particle size (*13*).

Emulsification is a very complex process that is influenced by several factors. Even a small change in any composition has a high effect on emulsion stability. The emulsifier concentration

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is one of several important factors that influence the emulsifying capacity to a great extent. For the oil-in-water beverage emulsion system, a low usage level of emulsifier can significantly reduce the cost of the beverage. The objectives of the present study were (a) to find out the minimum effective concentration of CFG to make a stable beverage emulsion and (b) to evaluate CFG-1 and 2 from coarse and fine corn fiber as an emulsifier and stabilizer of dilute beverage emulsions and determine their sugar linkage composition. By this latter information, we hope to define the structure/function relationships for CFG that can be used by future commercial manufacturers to make a consistent and well-characterized effective product for food and non-food applications.

MATERIALS AND METHODS

Materials. The oven-dried coarse and fine corn fiber samples were kindly provided by ADM Research. They were ground to a 20-mesh particle size using a Wiley mill, extracted with hexane to remove oil (*14*), and destarched by treating with Termamyl α-amylase (a gift from Novo Nordisk Bioindustrials, Inc., Danbury, CT) (*15*, *16*). Cold-pressed Valencia orange oil was obtained from Citrus and Allied Essences, Ltd., Belcamp, MD. The Polytron homogenizer (model PT 10/35) was purchased from Brinkmann, Westbury, NY, and the EmulsiFlex-B3 high-pressure homogenizer was purchased from Avestin, Inc. (Ottawa, Canada).

Isolation of CFG. CFGs were isolated from deoiled and destarched corn fiber according to the alkaline hydrogen peroxide procedure of Yadav et al. (*1*, *2*). In brief, deoiled and destarched corn fiber was mechanically stirred in a mixture of 0.1 M NaOH and 0.05 M $Ca(OH)_2$ solution containing 1 mequiv of each per gram of fiber in the extraction medium and boiled for 1 h. The residue obtained after centrifugation was resuspended in water, boiled for 5 min and centrifuged again. The combined supernatant was treated with H_2O_2 (0.1 g/g fiber), pH 11.5, at room temperature for 2 h, and then the pH was adjusted to 4.0−4.5 to precipitate hemicellulose A (Hemi. A). The supernatant was treated with two volumes of ethanol to precipitate Hemi. B (CFG-1) collected and dried in a vacuum oven at 50 °C.

The residue left after alkali extraction was further extracted with alkaline H_2O_2 (0.1 g/g fiber, pH 11.5) at a boiling temperature for 1.5 h. The residue was removed by centrifugation, and the pH of the supernatant was adjusted to 4.0−4.5 to precipitate Hemi. A. The CFG-2 was obtained from the supernatant by precipitating it with 2 vol of ethanol, collected, and dried in a vacuum oven as for CFG-1 described above.

Determination of Carbohydrate Composition. Sugars were analyzed by high-performance anion-exchange chromatography with pulsed amperometric detection (HPAEC-PAD) using methanolysis (*17*, *18*) combined with trifluoroacetic acid (TFA) hydrolysis. The gum samples to be analyzed were first dissolved in deionized water (1 mg/mL). An aliquot of 100 μL of this solution along with 100 nmol of myo-inositol (internal standard) were dried in a Teflon-lined screw cap glass vial by blowing with filtered nitrogen followed by drying in a vacuum oven at 50 °C overnight. These samples were methanolyzed with 1.5 M methanolic HCl in the presence of 20% (v/v) methyl acetate for 16 h, cooled to room temperature, and dried by blowing with filtered N_2 after adding five drops of *t*-butanol. The methanolyzed samples were hydrolyzed with 0.5 mL of 2 M TFA at 121 °C for 1 h and evaporated by blowing with filtered N_2 at 50 °C, and the residue was washed by sequential addition and evaporation of three aliquots (0.5 mL) of methanol. In three separate glass vials were placed 100, 300, and 500 nmol of a mixture of standard sugars containing fucose, arabinose, rhamnose, galactose, glucose, xylose, glucuronic acid, and galacuronic acid. Then, 100 nmol of myo-inositol (internal standard) was added to each vial, evaporated, and dried as above. These standard samples were also methanolyzed and hydrolyzed as described above and used for quantification.

Hydrolyzates were analyzed for neutral and acidic sugars by HPAEC-PAD using a Dionex ICS-2500 system that included a CarboPac PA10 column and guard column, a GP 50 gradient pump, an ED50 electrochemical detector utilizing the quadruple potential waveform (gold working electrode and pH reference electrode), an AS50 autosampler with a thermal compartment (30EC column-heater), and a PC10 pneumatic controller postcolumn addition system. The mobile phase consisted of isocratic 25 mM KOH eluant for 30 min followed by 100 mM KOH and 20 mM CH_3COOK for 10 min at a flow rate of 0.5 mL/min at ambient temperature. A 5-min column wash with 500 mM KOH followed by 15-min equilibration with 25 mM KOH at a flow rate of 1 mL/min at ambient temperature was required to yield highly reproducible retention times for the monosaccharides. The total run time was ca. 60 min. In order to minimize baseline distortion due to change in the pH of the eluant during monosaccharide detection by PAD, 730 mM KOH was added to the postcolumn effluent via a mixing tee.

Determination of Glycosyl Linkage Composition. Glycosyl linkage composition was determined by the CCRC at the University of Georgia, Athens, GA, by methylating pure CFG according to the method of Hakomori (*19*) following the detailed procedure described by York et al. (*20*). The permethylated samples were hydrolyzed with 2 M TFA (121 °C, 2 h), reduced with $NaBD_4$, and converted to their alditol acetates by using acetic anhydride and TFA. The partially methylated alditol acetate derivatives were analyzed by gas chromatography (GC) (Hewlett-Packard 5890) interfaced to a mass selective detector (MSD, electron impact ionization mode, Hewlett-Packard 5970). GC-MSD analysis was performed on a 30 m, Supelco 2330 bonded phase fused silica capillary column as described by York et al. (*20*).

Gum Solution. Stock gum solution, containing 6% (w/w) gum, 0.1% (w/w) sodium benzoate (a preservative), and 0.3% (w/w) citric acid was prepared by slowly adding the calculated amount of gum sample a little at a time with vigorous stirring to a solution of sodium benzoate and citric acid in water at room temperature and then gently stirring overnight to produce a hydrated, well-dissolved, and homogeneous solution (*2*). An adjustment for moisture and ash content in the sample was done during the gum solution preparation.

Emulsion Preparation. The samples for oil-in-water emulsions were prepared in triplicate for each sample at each concentration by taking 0, 42, 104, 208, and 1042 mg aliquot from the 6% stock gum solution (i.e., 0.00, 2.50, 6.25, 12.50, and 62.50 mg gum sample) in different vials and then adding sufficient deionized water to give 2.375 g of the solution. To the resulting solution, 125 mg of Valencia orange oil was added, which made a total of 2.5 g of final solution. No weighting agent was added to avoid the effect of such agent on the emulsification process. The solution was vortexed and then prehomogenized using a polytron bench top homogenizer equipped with a 12 mm diameter head (Brinkmann, Switzerland, PT 10/35) at 15 000 rpm for 30 s. The above prehomogenized emulsion was passed through the EmulsiFlex-B3 high-pressure homogenizer (Avestin, Inc., Canada) at 10 000 psi homogenization pressure three times. The resulting emulsion concentrate was diluted 31.25× to 78.125 g in a 10.0% (w/w) sucrose solution containing 0.1% (w/w) sodium benzoate and 0.3% (w/w) citric acid. Both the polytron homogenizer probe and the high-pressure homogenizer chamber were rinsed with ∼2.5 mL of sugar solution, 3× (saved from the total solution for this purpose). Each time, the polytron homogenizer probe was dipped into the solution and run for a few seconds, and then that solution was passed through the high-pressure homogenizer as above. After these three rinses, the high-pressure homogenizer was rinsed with one passage of air to blow the remaining traces of liquid out of the sample chamber. All three rinses were combined into the 75.625 g of total solution in a 125 mL glass bottle and set on a bench at room temperature.

After finding the optimum gum-to-oil ratio (0.05) to produce a stable emulsion, concentrates containing 6.25 mg of CFG and 125 mg of orange oil (0.05 gum-to-oil ratio) in the presence of 0.1% (w/w) sodium benzoate and 0.3% (w/w) citric acid were prepared in triplicate for each of four CFG samples, diluted in sugar solution, and set at room temperature as above.

Measurement of Emulsifying Properties by Turbidity Measurement. The emulsion stability was measured as turbidity (*12*) with modification as we explained in our previous article (*2*). $T = 2.303AD/l$, where T is the turbidity in reciprocal centimeters, A is the observed absorbance at 650 nm, D is the dilution factor, and l is the path length

of the cuvette in centimeters. The turbidity was determined immediately after preparing the dilute emulsion by measuring absorbance at a wavelength of 650 nm using a UV-1700 spectrophotometer (Shimadzu, Columbia, MA). The emulsion stability at each concentration was determined by emulsion breakage which was monitored by absorbance (loss of turbidity) measurement at 650 nm against a 10.0% sugar solution containing 0.1% sodium benzoate and 0.3% citric acid after 3 and 8 days. The emulsion breakage of the samples prepared with an optimum gum-to-oil ratio (0.05) was measured after 3, 8, 9, and 10 days.

Confocal Laser Scanning Microscopy (CLSM). The emulsions were viewed with a model TCS SP CLSM system integrated with a model IRBE optical microscope fitted with a 63× water immersion lens (Leica Microsystems, Exton, PA). Emulsions containing 0.05 (1:20) CFG and oil were prepared and diluted as described above. After standing at room temperature in sealed vials for 3 and 8 days, a milliliter aliquot from the bottom of the emulsion bottle was mixed with 5 μL of the hydrophobic dye Nile Red (0.1% in acetone) using a vortex. After standing for 20 min, a 10 μL drop of the dye mixture from each emulsion was placed in a glass-bottomed microwell dish (MatTek Corp., Ashland, MA) and spread into a thin film by the addition of a dry, clean 10 mm diameter coverslip, (degreased by sonication, first in acetone, then absolute ethanol). The oil droplets labeled with Nile Red were viewed by excitation of the dye in the sample with the 488 nm laser line of an argon laser, and images of the visible fluorescence were collected at 590−620 nm within single focal planes, each with an estimated thickness of 290 nm, using the LCS software of the microscope system. The size distribution of visible oil droplets in single focal planes was calculated from five digital images of each sample using Fovea Pro 3.0 (Reindeer Graphics, Ashville, NC): images were modified by changing the mode to grayscale, "surface" flattened, and the intensity was inverted before setting the lower and upper threshold limits and measuring selected features. The values for equivalent diameter were extracted and plotted using Microsoft Excel spreadsheets.

RESULTS AND DISCUSSION

Isolation of CFG. The CFG-1 was extracted from the destarched corn fiber sample by a mixture of NaOH (0.1 M) and Ca $(OH)_2$ (0.05 M), containing 1 mequiv of each alkali/gram of fiber in the reaction medium, at the boiling temperature and treating the alkaline extract with 0.1 g H_2O_2/g fiber (pH 11.5) at room temperature to bleach the product (*15*). This treatment released most of the CFG-1 linked through alkali labile phenolic or nonphenolic ester linkages or cross-linked by diferulic ester bridges (*21*). The CFG-2 is only extracted by alkali when H_2O_2 is also included during the second, sequential extraction. The exact association of CFG-2 to the cell wall is not well-known, but it may be strongly bound to the cell wall by a nonphenolic linkage, which becomes accessible to the alkaline H_2O_2 treatment. The amount of released CFG-1 and 2 fractions from coarse fiber were 29.0% and 8.0%, and that from fine fiber was 14.8% and 5.2%, respectively, of the total weight of the deoiled and destarched fiber (*4*).

The proximate analysis result of CFG-1 and 2 from coarse and fine fiber was reported in our previous publication (*4*). The moisture content of these gum samples ranged from 4.0 to 6.6%, which was high enough to justify adjustment for the moisture in emulsion preparation. The ash contents in all samples were very close to each other, ranging from 4.0 to 5.9%, and the correction for it was made during gum solution preparation. The protein content was comparatively higher in CFG-2 than CFG-1 from each corn fiber source. As expected, the average protein content (4.5%) of CFG-1 and 2 from fine fiber, which originates from the endosperm portion of the corn kernel, was higher than the average protein content of CFG-1 and 2 isolated from pericarp-originating coarse fiber (3.4%).

Sugar Composition. The CFGs from both coarse and fine fiber sources do not differ significantly in the general sugar composition (**Table 1**). They all have a typical arabinoxylan structure with an Ara/Xyl ratio ranging from 0.50 to 0.66, which is typical for arabinoxylan from corn bran (*22*). The low Ara/Xyl ratio (0.50) in CFG-1 from coarse fiber may be an indication of a less branched structure compared to that of the CFGs from the other sources. As reported in the literature, they contain about 29−36% Ara, 55−59% Xyl, 4−6% Gal, 0−2% Glc, and 3−5% GlcA, (*15*, *22*−*25*). The detection of any glucose probably indicates residual alkali-soluble starch or xyloglucan polymer left in the CFG preparation. The very low level of glucose (0.09−1.57%, **Table 1**) shows that the enzymatic treatment of CF by α-amylase to remove starch was effective.

Table 1. Carbohydrate Composition of CFG (mol %)

	coarse corn fiber		fine corn fiber	
sugars[a]	CFG-1	CFG-2	CFG-1	CFG-2
Ara	28.99	36.03	33.41	36.02
Gal	6.01	5.49	6.27	4.32
Glc	1.57	0.09	1.23	0.50
Xyl	58.61	55.26	54.74	54.90
GlcA	4.87	3.15	4.35	4.26
total	100	100	100	100

[a] Ara = arabinose; Gal = galactose; Glc = glucose; Xyl = xylose; GlcA = glucuronic acid.

Carbohydrate Linkage Composition. Methylation analysis data (**Table 2**) demonstrates that all CFG samples contain a similar arabinoxylan structure with a (1 → 4)-linked Xylp-backbone and arabinose in the side chain. The structural features are consistent with the reported arabinoxylan structure of maize bran (*22*), in which the 4-linked xylan backbone is highly substituted on position 2 and/or 3 by arabinose, xylose, and galactose residues. Arabinose was mainly found as a terminal residue and in 2-, 3-, or 5-linked positions. Xylose was found as a terminal residue and in 2- and/or 3-linked position in addition to its 1,4-linked xylan backbone. Galactose was mainly found as a terminal residue. All these CFGs are highly branched galactoglucuronoarabinoxylans containing only about 16−25% of total xylose in the backbone with no side chain (assuming that all 4-linked xylose residues belonged to the xylan backbone). The terminal Xyl residues represent about 4−9%, and the remaining 66−80% of total xylose residues were substituted. CFG-1 and 2 from fine fiber contain more terminal Ara and Gal than do the CFG-1 and 2 isolated from coarse fiber, indicating a more branched structure, but a solid conclusion regarding *t*-Ara content cannot be drawn, since there is always a possibility of losing a part of it during methylation and/or acid hydrolysis.

Emulsifying Properties. Generally, several steps are required to prepare a good emulsion. First the gum solution has to be prepared by slowly adding the weighed amount of gum to the rapidly stirred solution containing preservative and citric acid to make a completely dissolved solution. The gum solution is stirred for several hours or preferably overnight to completely hydrate the gum and make a very homogeneous solution. The next step is prehomogenization in which a precalculated amount of oil and homogeneous gum solution are taken and mixed together by using a high-speed polytron-type homogenizer to make a coarse emulsion or premix. During the coarse emulsion preparation, the oil phase is broken into oil droplets of less than 20 μm (*26*). The final and the most important step of the process is the homogenization under very high pressure. In this step, the coarse emulsion is introduced into the homogenization valve of the homogenizer at high pressure. Upon opening the homogenization valve, the high pressure forces the solution to

Table 2. Glycosyl-Linkage Composition of Neutral Sugars Present in CFG

		relative peak area %			
		coarse corn fiber		fine corn fiber	
alditol acetate derivative	deduced residue linkage	CFG-1	CFG-2	CFG-1	CFG-2
2,3,5-Me_3, Ara	terminal-Ara_f	8.7	8.0	19.7	20.2
3,5-Me_2, Ara	1 → 2-Ara_f	7.3	6.2	4.2	5.9
2,5-Me_2, Ara	1 → 3-Ara_f	7.5	6.8	5.4	4.3
2,3-Me_2, Ara	1 → 5-Ara_f(1→4-Ara_p)	8.1	5.7	9.6	2.6
2,3,4-Me_3, Xyl	terminal-Xyl_p	3.4	3.2	2.1	5.4
2,3-Me_2, Xyl	1 → 4-Xyl_p	10.2	12.7	13.1	12.9
3(2)-Me, Xyl	1 → 2(3),4-Xyl_p	28.0	33.9	27.7	29.6
Xyl	1 → 2,3,4-Xyl_p	22.3	19.3	10.0	9.8
2,3,4,6-Me_4, Gal	terminal-Gal_p	4.4	4.2	8.0	9.3

pass through a narrow valve at very high velocity, which creates agitation and cavitational forces that break the oil droplets into finer particles (*27*). For beverage emulsions, usually a two-stage homogenization (*13*, *28*) and high-pressure treatments (*29*) are preferred. In the present study, the prehomogenization step to prepare a crude emulsion was done by using a high-speed polytron homogenizer, which, upon further homogenization by passing through a high-pressure EmulsiFlex-B3 homogenizer (see Materials and Methods for detail) gave the finished concentrated emulsion.

The emulsifying properties of CFG were evaluated by the photometric method of Pearce and Kinsella (*12*), determining absorbency at 650 nm and calculating the turbidity and emulsion stability. This method is a simple but indirect measurement of emulsifying properties and is recommended for fast and routine analysis (*13*, *30*). The emulsion concentrates were diluted 31.25× in the sugar solution, and their absorbency was measured immediately (data not shown) and after 3 and 8 days by taking an aliquot of the solution from the bottom of the bottle. The turbidities of all solutions immediately after making dilute emulsions were very similar to each other, showing that solutions were very well homogenized and gave emulsions with very fine particle size regardless of the quality of the emulsifier. The turbidities of emulsions prepared without any emulsifying agent were as high as the turbidities of emulsions prepared in the presence of CFG emulsifiers (data not shown). However, the emulsions without emulsifier broke down in a day, and oil droplets started floating at the top of the solution. The emulsions prepared in the presence of the hydrocolloid (CFG), however, did not reach that stage so quickly.

The emulsion stabilities were studied by using 0.00, 0.02, 0.05, 0.10, and 0.50 gum-to-oil ratios (w/w) of each of the CFG-1 and 2 samples derived from coarse and fine fiber. The experiments with different oil-to-gum ratios were performed to determine a minimum amount of gum to make a stable emulsion and to compare an effectiveness of different CFG samples at that particular gum-to-oil ratio. Usually the beverage industries use a high stabilizer-to-oil ratio to make the stabilizer very effective at keeping the flavor oil droplets dispersed. But for a comparative study of different samples, to know the minimum concentration of stabilizer to be effective is essential. In general, within a certain range, the stability of an emulsion increases as the stabilizer-to-oil ratio increases. But, on the other hand, if the ratio of stabilizer to oil is lower than a certain range, emulsions may not be stable, and they may exhibit "creaming" or "ringing". It is also true that increasing the stabilizer-to-oil ratio above a certain range does not usually provide additional stability and only adds additional (unjustified) cost. In some cases, increasing levels above the optimum can actually decrease the stability of the emulsifier (*31*).

Figure 1 shows the turbidities of the diluted emulsions at gum-to-oil ratios of 0.00, 0.02, 0.05, 0.10, and 0.50 (w/w) for CFG-1 and 2 from coarse and fine corn fiber. The turbidities were measured after 3 and 8 days by taking an aliquot from the

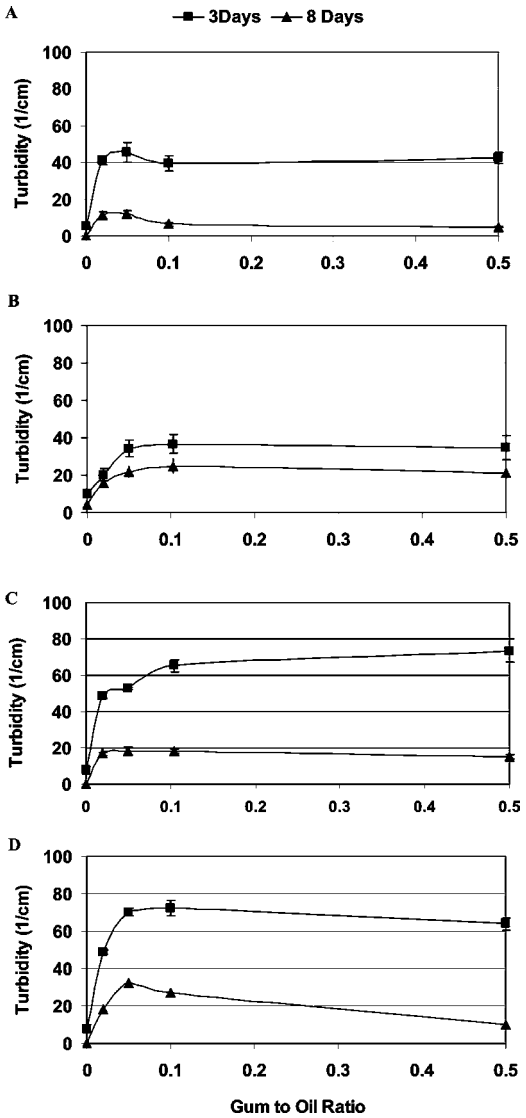

Figure 1. Emulsion stability (turbidity) of CFGs at room temperature with different gum-to-oil ratios: (**A**) CFG-1 from coarse fiber, (**B**) CFG-2 from coarse fiber, (**C**) CFG-1 from fine fiber, and (**D**) CFG-2 from fine fiber. Turbidity was measured by taking an aliquot from the bottom of the diluted solution after 3 and 8 days. Higher turbidity indicates a greater emulsifying capacity. Each plotted point is an average of three trials ± standard deviation.

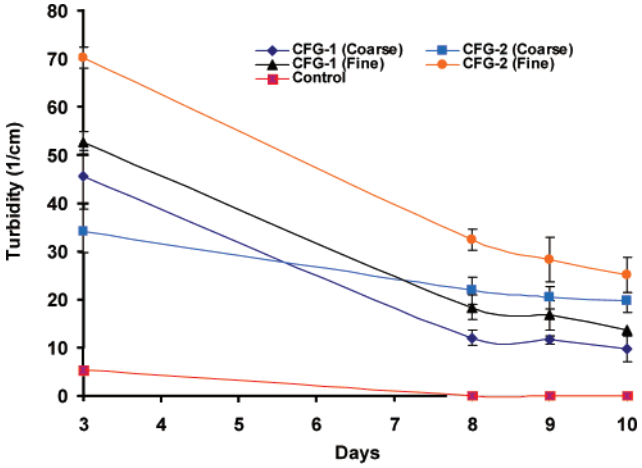

Figure 2. Emulsion stability (turbidity) of CFGs from coarse and fine fiber at room temperature with a 0.05 gum-to-oil ratio (w/w). Turbidity was measured by taking an aliquot from the bottom of the diluted solution after 3, 8, 9, and 10 days. Higher turbidity indicates a greater emulsifying capacity. Each plotted point is an average of three trials ± standard deviation.

clear droplet-depleted solution from the bottom of the bottles. Turbidities of emulsions containing CFG-1 from coarse fiber increased as the gum-to-oil ratio increased to 0.05, decreased at a ratio of 0.1, and stayed relatively constant up to a ratio of 0.5 on both the third and eighth days. In a similar way, the turbidities of emulsions containing CFG-2 from coarse fiber increase continuously up to a 0.1 gum-to-oil ratio and, after that, remains without any turbidity change on both the third and eighth days. This pattern of increase in turbidities follows for both CFG-1 and 2 from fine corn fiber up to a gum-to-oil ratio of 0.05 for both the 3 and 8 day study, but in the 3 day studies, there is a further gradual increase in stability as ratios of gum to oil increase to 0.5 for CFG-1 from fine fiber. In the case of CFG-2 from fine fiber, the turbidities increase upon increasing the gum-to-oil ratio to 0.05 like other gum samples, but after that, they decrease slightly on both days. Thus, these results clearly demonstrate that emulsion stabilities of all these CFG samples definitely increase with the higher concentration of gum up to about a 0.05 gum-to-oil ratio. After that concentration, the emulsion stability curve usually flattens or does not change significantly. These results agree with the findings of Einhorn-Stoll (*13*) and Ishii et al. (*32*) that higher emulsifier concentration favors the quick stabilization of the oil interface and reduces recoalescence of the oil droplets formed during the homogenization process up to a certain concentration increase. Depending upon the quality of the emulsifier, after a certain concentration, there is no effect of increasing concentration on the emulsion stability.

Figure 2 shows the turbidities of the emulsion containing 6.25 mg of CFG and 125 mg of oil (gum-to-oil ratio = 0.05) after 3, 8, 9, and 10 days of diluted emulsion preparation. The turbidity of each sample decreased from the third to the eighth day, and after that they became almost constant. The turbidity of the emulsion containing no emulsifier (control) reached zero on the eighth day. The difference in the emulsion stabilities of CFG-1 and 2 from both coarse and fine corn fiber is visible. After the eighth day, the emulsion stability of CFG-2 looks superior to that of the corresponding CFG-1 from each respective source. This is consistent with our previous finding that CFG-2 is a better emulsifier than the corresponding CFG-1 (*2*). CFG-2 in this case (and in our previous studies) almost always has a higher protein content that the corresponding CFG-1,

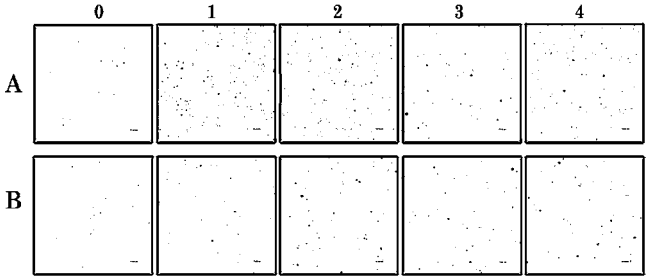

Figure 3. Confocal micrographs of emulsion containing a 0.05 gum-to-oil ratio (w/w) after (**A**) 3 and (**B**) 8 days: (0) Control containing no emulsifier, (1) CFG-1 from coarse fiber, (2) CFG-2 from coarse fiber, (3) CFG-1 from fine fiber, and (4) CFG-2 from fine fiber.

Table 3. Particle Size[a] Determined by Confocal Microscopic Imaging

	particle size (μm)	
sample	3 days	8 days
CFG-1 (coarse)	0.6 ± 0.4	0.7 ± 0.5
CFG-2 (coarse)	0.6 ± 0.4	1.0 ± 0.9
CFG-1 (fine)	0.8 ± 0.6	0.8 ± 0.7
CFG-2 (fine)	0.7 ± 0.5	0.6 ± 0.5

[a] Average particle size from five images ± standard deviation.

suggesting, but not proving that the protein may be involved in its emulsification properties. We also found in our previous article (*4*), that CFG-2 from each fiber source has more total lipid associated with it than the corresponding CFG-1, suggesting that lipid may also play a functional role in CFG's ability to stabilize oil-in-water emulsions. This finding is similar to that of our previous work on gum arabic (*33*), where we conclusively showed in that system that, in addition to protein, lipid components are key for the emulsion stability of oil-in-water emulsion systems. If CFG and gum arabic function similarly, the lipid portion of CFG in combination with hydrophobic protein adsorbs on the oil surface and acts as an anchor to the whole molecule, and the big hydrophilic polysaccharide portion extends into the aqueous phase and stabilizes the oil droplets by steric and electrostatic repulsion. Studies remain to be done to conclusively prove this mechanism.

A CLSM study of the emulsion containing CFG was performed after 3 and 8 days to further examine the emulsions and their stability. CLSM observation of the diluted emulsion of all four CFG samples clearly indicated emulsions with particle size of 1 μm or lower, but it did not show any remarkable differences in droplets sizes for the different CFG samples (**Figure 3** and **Table 3**). The difference in the particle size might be below the detected particle size which was cut off in this microscopic study. This microscopic study of emulsion in which tiny oil droplets are stained with a fluorescent dye (Nile Blue) gives a very good picture of the emulsion particles, but the minor particle size difference is not well differentiated. The emulsion stability difference among the four CFG samples is clearly demonstrated by the difference in their turbidities during 3−10 days of emulsion stability studies (**Figure 2**).

Conclusions. The present study has shown that only 0.25% of CFG is required to make a stable emulsion containing 5% orange oil under the used experimental conditions. Emulsions prepared with CFG-2 from each fiber source have comparatively higher stability than the corresponding CFG-1 from each source in a 10-day emulsification study at room temperature. The emulsion stability investigation by CSLM measurement showed that CFG-1 and 2 from both coarse and fine fiber sources made stable emulsions with an average particle size of less than

1 μm. Glycosyl linkage determination by methylation analysis suggests that CFG has a typical highly branched arabinoxylan structure with only 16−25% of 1→4-linked xylose residues in the xylan backbone without any substitution (or side chains) at the O-2 and/or O-3 position.

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