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A new corn fiber gum polysaccharide isolation process that preserves functional components☆

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ABSTRACT

Corn fiber gum (CFG) is a hemicellulose (arabinoxylan)-enriched fraction obtained by the extraction of corn bran/fiber using a mild alkaline hydrogen peroxide process. The unique polysaccharide, CFG, with its low solution viscosity has been proposed as a stabilizer for oil-in-water emulsions. We have verified that in some model systems, CFG can out-perform the "gold standard" emulsifier, gum arabic. Our results have also shown that "pure" CFG fractions often contain considerable amounts of associated lipids, phenolic acids and proteins which contribute to its emulsifying properties. The extraction of CFG with alkaline hydrogen peroxide was investigated using different combinations of alkali concentration and time to identify the optimum extraction condition to retain its functional groups (protein, lipids and phenolic acids). The pure CFG prepared by this process was hydrolyzed with 1.5 N methanolic KOH at 70 °C for 1 h to release hydroxycinnamic acids (p-coumaric and ferulic) and lipids. The total lipid was extracted with chloroform/methanol, evaporated and quantified. The released phenolic acids were identified and quantified using HPLC with detection by both UV and evaporative light-scattering detection (ELSD). The protein content was determined by an AACC approved combustion method. The total lipids, phenolic acids and protein content in CFGs isolated with lower alkali concentration for a shorter time was comparatively higher than CFGs isolated with higher alkali concentration for a longer time. The presence of these phenolic acids, lipids and protein in CFG may contribute to its excellent emulsifying properties and may combine to give improved chemical, physical, and even nutritional properties. Understanding these critical structural elements required for optimal emulsification properties will allow future commercial producers of CFG to provide consistent quality and functionality in their products.

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1. Introduction

Corn fiber (CF), the most abundant low-valued coproduct of the industrial corn wet-milling process contains a large percentage of valuable hemicelluloses. The structural and chemical characterization of corn fiber hemicelluloses reported by many investigators (Montgomery & Smith, 1957; Montgomery, Smith, & Srivastava, 1957; Whistler & BeMiller, 1956; Whistler & Corbett, 1955, 1956; Yadav, Johnston & Hicks, 2007), have shown that it has an arabinoxylan structure, consisting of a β-1,4 linked D-xylopyranosyl backbone with α-L-arabinofuranosyl substituents attached at positions 2 and/or 3 and with glucuronic acid substituents attached primarily at position 2. The ferulic acid and other hydroxycinnamic acids can be attached at 5 position of arabinose. The arabinoxylan (AX) isolated from corn fiber by an alkaline hydrogen peroxide technology is called corn fiber gum, CFG (Doner & Hicks, 1997; Yadav, Johnston, Hotchkiss, & Hicks, 2007; Yadav, Fishman, Chau, Johnston, & Hicks, 2007; Yadav, Cooke, Johnston & Hicks, 2010). The fiber fractions from the kernel's pericarp and endosperm produced by the corn-wet milling industry are called "coarse" and "fine" fibers, respectively and collectively they are also called "white fiber". CFGs isolated from both coarse and fine corn fiber contain functional protein and lipid and also nutraceutical phenolic compounds (Yadav, Moreau & Hicks, 2007).

There is enough evidence to suggest that corn AX polymers in intact corn kernels are cross-linked to each other and/or other cell wall polymers through dehydrodiferulate ester bridges (Chanliaud, Saulnier, & Thibault, 1994; Saulnier, Crepeau, Lahaye, & Thibault, 1999; Saulnier, Marot, Chanliaud, & Thibault, 1995; Saulnier, Vigouroux, & Thibault, 1995) and/or dehydrotriferulate ester bridges (Funk, Ralph, Steinhart, & Bunzel, 2005; Rouau et al., 2003). It is well known that corn fiber contains ferulic acid (4-hydroxy-3-methoxycinnamic acid) together with a small amount of p-coumaric (4-hydroxy-cinnamic), dehydrodiferulic and dehydrotriferulic acids, which are covalently bound to cell wall hemicelluloses (Funk et al., 2005; Ohta, Yamasaki, Egashira, &

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Sanada, 1994; Rouau et al., 2003; Saulnier, Vigouroux, et al., 1995). Phenolic acids such as ferulate and diferulate, bound to corn arabinoxylan, contribute to the cross linking of xylan to lignin chains according to Lapierre, Pollet, Ralet, and Saulnier (2001). Oligosaccharide fragments with arabinosyl units esterified at primary hydroxyl groups with ferulic acids obtained by enzymatic (Ohta et al., 1994) and partial acid (Saulnier, Vigouroux, et al., 1995; Yoshida et al., 1990) hydrolysis of corn fiber hemicelluloses further supports the presence of phenolic acids on corn AX. Phenolic acids are believed to be linked by an ester linkage to AX, as they can be easily released from cell walls by alkali treatment. These hydroxycinnamic acids have been suggested to possess many health promoting properties. The ester-linked hydroxycinnamic acids can be released from the cell walls into the colon by the action of bacterial esterases and they may be absorbed or converted into other compounds by colonic bacteria (Karoon, Faulds, Ryden, Robertson, & Williamson, 1997). They may also act as antimutagens to reduce the risk of cancer. The abilities of ferulic, p-coumaric and dehydrodiferulic acids to protect against different types of mutations in a bacterial system have been studied and also compared with the related compound curcumin (Ferguson, Lim, Pearson, Ralph, & Harris, 2003).

These phenolic acids (ferulic and p-coumaric acids) are well studied naturally occurring antioxidants (Cuvelier, Richard, & Berset, 1992) and the antioxidative activity of corn bran hemicellulose containing hydroxycinnamic acids has also been reported (Graf, 1992; Ohta et al., 1994). Previously, we reported the importance of lipids on the stabilizing effect of gum arabic in oil-in-water beverage emulsion systems (Yadav & Nothnagel, 2007; Yadav, Johnston, Hicks, & Nothnagel, 2006). Our previous studies also indicated that CFG was as good as gum arabic or even a superior emulsifier in such oil-in-water emulsion systems (Yadav et al., 2006). The objective of the present investigation is to develop optimum CFG isolation conditions to preserve the functional (protein and lipid, known for emulsion stabilizing activity) and nutraceutical (phenolic compounds, known to be antioxidants and antimutagen agents) components associated with it.

2. Materials and methods

2.1. Materials

Wet milling pericarp fiber (WPF, also called "coarse fiber") and wet milling endosperm corn fiber (WEF, also called "fine fiber") samples were specially collected and kindly provided by ADM Research (North America) and wet pericarp and endosperm corn fiber (WPEF, also called simply "corn fiber" and used as the major ingredient in Corn Gluten Feed) sample by Cargill Central Research (Minneapolis, MN). They were oven dried by the suppliers before shipping. Fiber samples were ground to a 20-mesh particle size using a Wiley mill and extracted with hexane to remove oil (Moreau, Powell, & Hicks, 1996). Starch was removed from the 20-mesh de-oiled fiber by treating with heat stable Termamyl α-amylase (Novozymes, Inc., Davis, CA) (Doner, Chau, Fishman, & Hicks, 1998). All the HPLC standards were obtained from Sigma Chemical Co., St. Louis, MO.

2.2. Standard proximate analyses

Moisture, protein ($N \times 6.25$) and ash contents of all CFG samples were determined using "AACC Approved Methods" 44-19, 46-30 and 08-01 respectively (AACC International, 1995).

2.3. Isolation of corn fiber gum

CFGs were isolated from de-oiled and de-starched corn fiber by previously reported alkaline hydrogen peroxide technology (Yadav, Cooke, et al., 2010) with some modification. The following three kinds of fibers were used for CFG isolation: (a) wet milling pericarp fiber, WPF (b) wet milling endosperm fiber, WEF and (c) wet milling pericarp and endosperm fiber, WPEF. The isolation was done with 2, 4 and 8 meq sodium hydroxide and calcium hydroxide alkali mixture (equal amount of each) per gram of fiber by heating at 100 °C for 15 min or 1 h. In brief, de-oiled and de-starched corn fiber (25 g) was mechanically stirred into water (0.5 L) and 2, 4 or 8 meq of alkali mixture and 21 mL of 30% H_2O_2 were carefully added in an open beaker in a fume hood. The mixture was boiled with efficient mechanical stirring for 15 min or 1 h. After cooling the hot reaction mixture by stirring at room temp for an additional half an hour, it was centrifuged at $6000 \times g$ for 20 min and the supernatant was separated from the residue by decantation. The pH of the alkaline H_2O_2 extract (supernatant) was then adjusted to 4.0–4.5 by adding Conc. HCl to precipitate Hemicellulose A (acid-insoluble arabinoxylan, "Hemi. A"), which was collected by centrifugation at $10{,}000 \times g$ for 30 min. Two volumes of ethanol were gradually added to the supernatant with stirring to precipitate the major arabinoxylan fraction, Hemicellulose B, or "Hemi. B", (CFG). The CFG was allowed to settle out as a white flocculent precipitate at the bottom of the beaker for 10–15 min. The clear alcohol/water mixture above the precipitate was removed by decantation. The white flocculent precipitate was transferred into another beaker, stirred in 100% ethanol and filtered under vacuum. The white residue obtained on the Buchner funnel was washed with 100% ethanol and dried in a vacuum oven at 50 °C overnight.

The residue left after alkaline H_2O_2 extraction was suspended into water, pH adjusted to 5.5–6.0 and stirred for about 15 min. The undissolved residue was collected by vacuum filtration using Whatman filter paper number 1, rinsed with 100% ethanol and dried in vacuum oven. This final water insoluble residue is called cellulose/arabinoxylan (CAX).

2.4. Determination of sugar composition

Sugars were analyzed by HPAEC-PAD (Zhao et al., 2008) after hydrolyzing CFG into monosaccharides by methanolysis combined with TFA treatment as explained in detail by Yadav, Johnston and Hicks (2007).

2.5. Methanolic KOH hydrolysis and extraction of total lipid with chloroform/methanol

CFGs were hydrolyzed using a strong alkaline solution, 1.5 M methanolic KOH by modified technique of Moreau and Hicks (2004) to release all phenolic acids and lipids and extracted with chloroform and methanol (Bligh & Dyer, 1959). For hydrolysis, CFG (0.5 g) was placed in a screw cap glass tube (55 mL, 25 mm × 150 mm), and 10 mL of 1.5 M methanolic KOH and 500 μL water were added to completely dissolve the sample. The tubes were sealed with Teflon lined screw caps and boiled by immersing in a water bath at 70 °C with stirring for 1 h. After cooling the tubes to room temperature, 6 mL methanol and 8 mL chloroform were added to each tube and mixed well. Then they were centrifuged at $70 \times g$ for 15 min and filtered through a Whatman GF/A glass filter paper (Whatman Laboratory Products, Clifton, NJ) fitted in a Buchner funnel, with a gentle vacuum. The pellet retained in the glass tube was re-suspended in 2 mL of 2:1 methanol and chloroform, mixed well and filtered through the same filtration set up to collect the filtrate in the same tube. The residue on the filter paper was rinsed with 1 mL of 2:1 methanol and chloroform and collected in the same tube again. To the combined filtrate (total 27 mL containing 18 mL methanol and 9 mL chloroform), 8.5 mL of water was added to make up the total volume of water 9 mL. The combined solution was acidified to pH 2–3 with 6 M HCl and 9 mL chloroform was added to

maintain 2:2:1 (v/v) methanol, chloroform and water ratio, which helps for a good phase separation (Bligh & Dyer, 1959). The reaction mixture was mixed well and centrifuged at 70 × *g* for 10 min for complete phase separation. The lower layer (chloroform layer) was collected in a clean vial, evaporated at 50 °C under a stream of filtered nitrogen and weighed.

2.6. Phenolic acid analysis

The phenolic acids (ferulic and p-coumaric) were analyzed by a HPLC system containing both UV and evaporative light-scattering detectors (ELSD). p-Coumaric and ferulic acids were identified by both detection systems, but they were quantified by UV absorption at 320 nm by comparing with the peak area of known amount of standard p-coumaric acid and ferulic acid. HPLC analyses of free ferulic and p-coumaric acids were performed on a Hewlett Packard Model 1100 HPLC (Agilent Technologies, Palo Alto, CA) with an autosampler, and detection via two detectors in series, with the effluent first entering a Hewlett Packard Model 1100 Diode Array UV-visible detector, and then entering a Sedex Model 55 Evaporative Light Scatter Detector, ELSD (Richard Scientific, Novato, CA) operated at 40 °C, and nitrogen as a nebulizing gas, at a pressure of 2.0 bar. The HPLC column was a LiChrosorb 7 micron DIOL stationary phase (3 mm × 100 mm, packed by Varian/Chrompack, Walnut Creek, CA) and the isocratic mobile phase was hexane/isopropanol/acetic acid, 75.0/24.9/0.1 (v/v), at a flow rate of 0.5 mL/min. All experiments were repeated at least two times and the results presented are the means of 3 analyses.

3. Results and discussion

3.1. Chemical extraction of corn fiber gums

From the de-oiled and de-starched CF, CFG was extracted with 2, 4 or 8 meq of alkali mixture (NaOH and $Ca(OH)_2$) per gram fiber at pH 11.5 in presence of 0.25 g H_2O_2/g fiber by boiling the reaction mixture for 15 min or 1 h. The CFG extraction was done from three fiber sources (a) wet pericarp fiber (WPF), (b) wet endosperm fiber (WEF) and (c) wet pericarp and endosperm fiber (WPEF) with three alkali concentration (2, 4 or 8 meq/g fiber) at two different incubation times (15 min or 1 h) to see the effect of alkali concentration and heating time on CFG yield and its functional components retention. The extraction procedure is a slight modification of our previously reported CFG isolation method (Yadav, Cooke, et al., 2010). The oil present in CF was removed by hexane extraction (Moreau et al., 1996) to avoid its oxidation product in CFG. The starch from de-oiled fiber was removed by boiling with heat stable α-amylase to avoid alkali soluble starch contamination in CFG. The yield of CFG from WPF, WEF and WPEF varies from 29.6% to 32.8%, 16.0% to 21.2% and 25.6% to 39.2% respectively from de-oiled and de-starched corn fiber (Table 1). The yield of CFG from WPF is higher than the endosperm originating fiber (WEF) as previously reported (Yadav, Cooke, et al., 2010) due to the higher percentage of starch in WEF than in WPF. It looks clear from Table 1 that there is not a big change in CFG yield due to increase in alkali concentration or incubation time. Thus it certainly indicates that more that 2 meq/g fiber alkali concentration and longer than 15 min incubation time in presence of 0.25 g H_2O_2/g fiber is not essential in the extraction medium.

3.2. Moisture and ash contents

Moisture content in CFG samples was determined, which varied from about 3.83% to 7.33%. These moisture percent was subtracted from the moist sample and calculation for the ash content was done on a totally dry weight basis to show the purity of CFG. The ash content in WPF (3.22–5.16%) and WPEF (3.40–4.70%) (Table 2) are in the range reported previously (Yadav et al., 2006). Similarly WEF has about 4.54–6.34% ash which is close to the value found in a similar sample (Yadav, Cooke, et al., 2010). The change in the extraction conditions does not have any remarkable effect in CFG ash content.

3.3. Sugar composition of corn fiber gums

In most cases, the CFGs isolated using three different alkali concentrations by boiling for 15 min or 1 h do not differ significantly in their general sugar composition (Table 3). They all have a typical arabinoxylan structure with a similar Ara/Xyl ratio containing about 30–45% Ara, 40–57% Xyl, 2–9% Gal, 0–4% Glc, 2–4% GlcA and a trace amount of Rha as previously reported by many investigators (Hespell, 1998; Saulnier, Marot, et al., 1995; Whistler & BeMiller, 1956; Yadav, Johnston & Hicks, 2007; Yadav, Johnston, Hotchkiss, et al., 2007). This observation clearly indicates that neither of the isolation conditions (high alkali concentration or longer incubation time) has any adverse effect on the typical carbohydrate composition of CFG. One sugar component that appeared to vary significantly was glucose, particularly at the longer reactions times and higher alkali concentrations with the WEF and WPEF. Starch was removed by the enzymatic treatment of corn fiber with α-amylase prior to CFG isolation. So the presence of glucose in CFG indicates either the presence of α-amylase-resistant residual alkali soluble starch or xyloglucan polymer present in isolated CFG. Regardless of its origin, it appears that CFG produced from WEF using 8 meq of alkali and that produced from WPEF using either 4 or 8 meq of alkali is virtually devoid of this monosaccharide, meaning that any co-mingled glucose-containing polysaccharide must have been removed with the more drastic conditions.

3.4. Protein content of corn fiber gums

The protein content of CFG isolated from three fiber sources with three alkali concentrations (2, 4 or 8 meq/g fiber) at two different incubation times is shown in Fig. 1. The protein content in CFG isolated from WEF (Fig. 1B) is higher than the CFG isolated from WPF or WPEF. This supports the previous finding that fiber obtained from corn endosperm contains more protein than the fiber from corn pericarp (Yadav, Johnston & Hicks, 2007; Yadav, Johnston, Hotchkiss, et al., 2007). The protein content in CFG isolated with 2 meq alkali mixture/g fiber for 15 min incubation time from all three fiber sources is higher than CFG isolated with higher alkali concentration and longer incubation time. As the alkali concentration is increased, we see a gradual decrease in the protein content in CFG form all fiber sources. The percent of protein is higher in CFG isolated with 15 min than 1 h incubation time from all fiber sources except CFG extraction with 4 meq alkali mixture/g fiber from WPF in which their yields are almost similar.

It is well documented that protein plays an important role for the emulsifying activities of naturally occurring emulsifiers, gum Arabic (Dickinson, 2003; Randall, Phillips, & Williams, 1988) and CFG (Yadav, Johnston & Hicks, 2007; Yadav, Johnston, Hotchkiss, et al., 2007; Yadav, Cooke, et al., 2010; Yadav, Nicholas, Onwulata & Hicks, 2010; Yadav, Strahan, Mukhopadhyay, Hotchkiss & Hicks, 2011). The hydrophobic protein associated with carbohydrate polymer makes a hydrophobic head and position to adsorb on the oil–water interface of emulsions and the hydrophilic carbohydrate molecules extends into the aqueous phase to stabilize the oil dispersion (Garti, 1999). Thus the CFG extraction conditions which preserves the highest percent of its protein is important for making a functional CFG with higher emulsion stabilizing capacity.

Table 1
Yields[a] of corn fiber gum isolated under different conditions (wt.%).

Sample	Alkali concentration					
	2 meq/g fiber		4 meq/g fiber		8 meq/g fiber	
	15 min[b]	1 h[b]	15 min[b]	1 h[b]	15 min[b]	1 h[b]
Wet milling pericarp fiber	32.8	30.0	36.0	30.0	29.6	31.2
Wet milling endosperm fiber	16.0	21.2	17.2	18.8	18.8	18.4
Wet milling pericarp and endosperm fiber	26.0	29.2	39.2	32.8	25.6	32.4

[a] Based on the amount of de-oiled and de-starched corn fiber.
[b] Isolation time at 100 °C.

Table 2
Moisture and ash content of corn fiber gum samples.[a]

Alkali concentration	Isolation time at 100 °C	Moisture	Ash
Wet milling pericarp fiber			
2 meq/g fiber	15 min	4.77	5.05
	1 h	5.26	3.22
4 meq/g fiber	15 min	4.0	4.09
	1 h	5.91	4.84
8 meq/g fiber	15 min	5.42	5.16
	1 h	5.93	4.72
Wet milling endosperm fiber			
2 meq/g fiber	15 min	3.83	5.85
	1 h	6.56	4.54
4 meq/g fiber	15 min	4.67	5.64
	1 h	5.78	4.51
8 meq/g fiber	15 min	5.59	6.13
	1 h	6.75	6.34
Wet milling pericarp and endosperm fiber			
2 meq/g fiber	15 min	5.25	3.55
	1 h	5.44	3.92
4 meq/g fiber	15 min	4.44	3.64
	1 h	5.98	3.40
8 meq/g fiber	15 min	6.01	4.70
	1 h	7.33	4.54

[a] Wt. percent based on the dry weight of de-oiled and de-starched CFG samples.

Table 3
Sugar composition of corn fiber gums isolated under different conditions (mol%).

Samples	Rha	Ara	Gal	Glc	Xyl	GlcA	Total
CFG (WPF)							
2 meq, 15 min	1.04	30.10	7.45	4.09	53.40	3.91	100.00
2 meq, 1 h	0.77	32.05	8.21	2.52	52.82	3.64	100.00
4 meq, 15 min	0.83	31.91	7.95	2.00	53.49	3.81	100.00
4 meq, 1 h	0.64	30.86	8.20	2.30	54.66	3.34	100.00
8 meq, 15 min	0.53	31.13	7.89	3.34	54.22	2.88	100.00
8 meq, 1 h	0.97	31.40	8.57	2.55	53.38	3.14	100.00
CFG (WEF)							
2 meq, 15 min	2.21	36.46	6.89	1.98	48.01	4.46	100.00
2 meq, 1 h	2.23	35.36	7.71	3.66	47.21	3.83	100.00
4 meq, 15 min	2.32	37.11	8.04	1.55	47.47	3.50	100.00
4 meq, 1 h	2.39	34.69	8.30	3.97	47.11	3.55	100.00
8 meq, 15 min	2.58	35.28	2.38	0.21	55.88	3.66	100.00
8 meq, 1 h	2.40	35.78	2.94	0.11	55.25	3.52	100.00
CFG (WPEF)							
2 meq, 15 min	0.95	45.25	7.80	0.58	41.71	3.71	100.00
2 meq, 1 h	1.39	46.79	7.82	0.28	40.39	3.33	100.00
4 meq, 15 min	0.81	34.76	4.92	0.00	56.50	3.01	100.00
4 meq, 1 h	0.53	39.61	3.89	0.03	54.01	1.93	100.00
8 meq, 15 min	0.58	40.00	3.52	0.00	54.10	1.80	100.00
8 meq, 1 h	0.56	39.10	3.70	0.01	54.87	1.76	100.00

Note: Rha, rhamnose; Ara, arabinose; Gal, galactose; Glc, glucose; Xyl, xylose; GlcA, glucuronic acid.

3.5. Total lipid content of corn fiber gums

The CFG samples were hydrolyzed with strong solutions of alkali in methanol by incubating at 70 °C for 1 h to cleave associated lipids. The alkaline hydrolysis of CFG by treating with 1.5 KOH in methanol and about 5% of water at 70 °C for 1 h releases a considerable amount of lipids including phenolic acids (Yadav, Moreau, et al., 2007). The hydrolyzed total lipids were extracted with chloroform/methanol, evaporated and its yield in CFG was calculated.

The lipid content in CFGs isolated from three fiber sources with 2, 4, and 8 meq alkali mixture/g fiber by heating the reaction mixture for 15 min or 1 h is shown in Fig. 2. The amount of lipid (mg/g) in CFG isolated from WPF decreases gradually as alkali

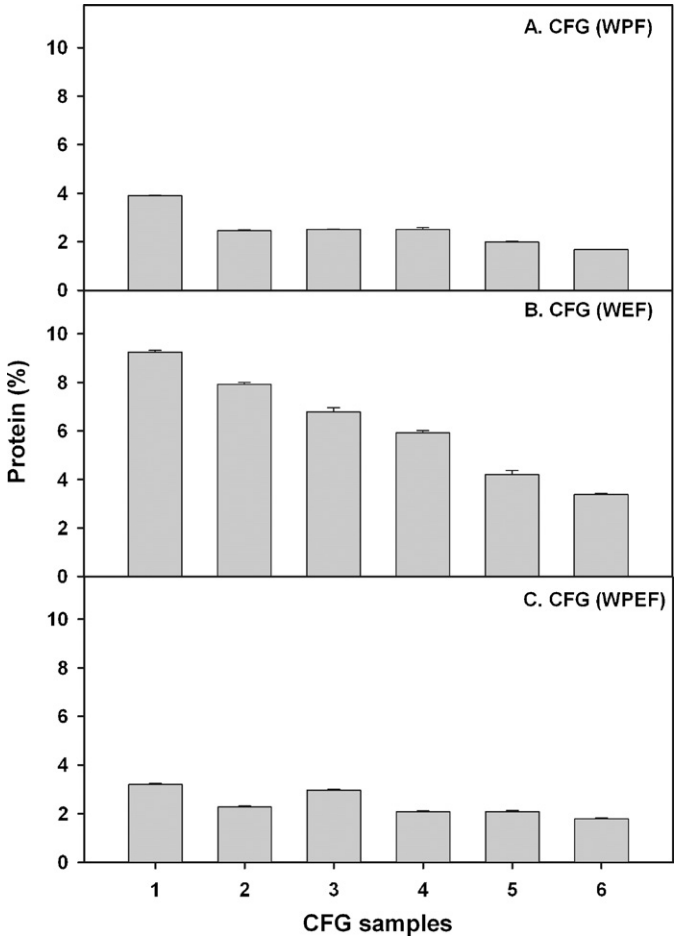

Fig. 1. Protein content of corn fiber gum isolated with 2, 4 or 8 meq alkali by heating at 100 °C. Alkali concentration and isolation time: (1) 2 meq, 15 min; (2) 2 meq, 1 h; (3) 4 meq, 15 min; (4) 4 meq, 1 h; (5) 8 meq, 15 min; (6) 8 meq, 1 h. Data are an average of six trials ± standard deviation.

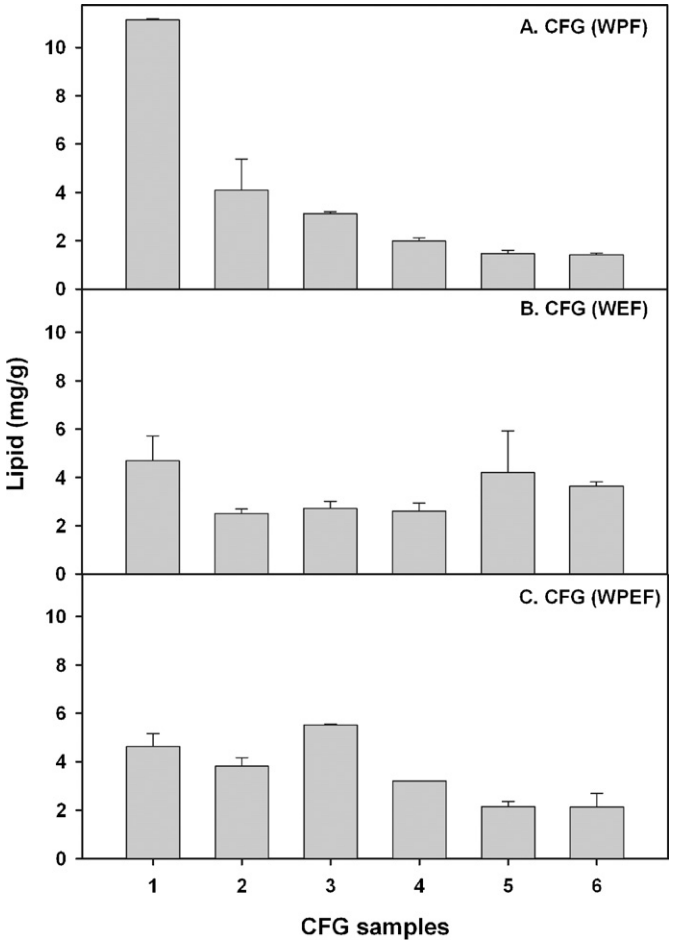

Fig. 2. Lipid content of corn fiber gum isolated with 2, 4 or 8 meq alkali by heating at 100 °C. Alkali concentration and isolation time: (1) 2 meq, 15 min; (2) 2 meq, 1 h; (3) 4 meq, 15 min; (4) 4 meq, 1 h; (5) 8 meq, 15 min; (6) 8 meq, 1 h. Data are an average of three trials ± standard deviation.

concentration and its extraction time increases showing that they are linked to (or at least associated with) arabinoxylan by an alkali susceptible ester linkage. Thus a low alkali concentration (2 meq/g) and short heating time (15 min) is required to preserve lipid on CFG during its isolation from WPF. But the same low alkali concentration and short heating time is not the optimum condition for CFG isolation from WEF and WPEF for maximum preservation of lipid. In case of WEF, we do see the highest lipid content in the CFG which is isolated either with 2 meq alkali/fiber for 15 min. But in the case of WPEF, 4 meq alkali and 15 min incubation time produces CFG which has a slightly higher lipid content than CFG isolated with 2 meq alkali for 15 min incubation. Thus it seems that 2 meq alkali and 15 min incubation is comparatively a favorable extraction condition for preserving its lipid but it is not true for fiber from all sources.

3.6. Phenolic acids content of corn fiber gums

The alkali-released ferulic and p-coumaric acids of CFG were analyzed by HPLC. HPLC chromatograms of standard p-coumaric (*trans*-p-coumaric) and ferulic (*trans*-ferulic) acids obtained with UV detection were used for identification and quantification. The UV detector peaks in CFG samples were identified by comparing their retention times (T_r) with standard phenolic acids (*trans*-p-coumaric and *trans*-ferulic).

The yield of ferulic and p-coumaric acids present in CFGs obtained from three fiber sources with 2, 4, and 8 meq alkali mixture/g fiber by heating the reaction mixture for 15 min and 1 h are shown in Figs. 3 and 4 respectively. The amount of both ferulic and p-coumaric acids in CFGs extracted from all three fiber sources by 2 meq alkali mixture/g fiber heating at 100 °C is the highest in comparison to CFGs extracted with higher alkali concentration and longer heating time. The amount of ferulic acid in CFGs extracted from WPF, WEF and WPEF with 2 meq alkali mixture/g fiber for 15 min heating is above 600, 275 and 200 μg/g CFG sample respectively. When these fibers are extracted with the same alkali concentration (2 meq alkali mixture/g fiber) but heating for 1 h, the ferulic acid content in CFGs from WPF and WPEF decrease slightly but the amount of this phenolic acid in CFG from WEF decrease dramatically to below 25 μg/g showing a big effect of heating for a longer than 15 min. The amount of ferulic acid in CFGs extracted with 4 and 8 meq alkali mixture/g fiber by boiling either for 15 min or 1 h is below 100 μg/g sample and in some cases even below 20 μg/g CFG sample. The amount of p-coumaric acid is high in CFG extracted with low alkali concentration and it follows the pattern of ferulic acid yield except isolation from WPF and WPEF with 2 meq at 1 h heating time. In this isolation condition, irrespective of ferulic acid, its yield is higher with 1 h incubation in comparison to 15 min. But the sum of these two phenolic acids with 15 min heating is higher than 1 h incubation, which confirms that an isolation of CFG for a shorter time preserves a high percent of total phenolic acids.

These results clearly indicate that for preserving the nutraceutical phenolic compounds, CFG needs to be extracted with a low alkali

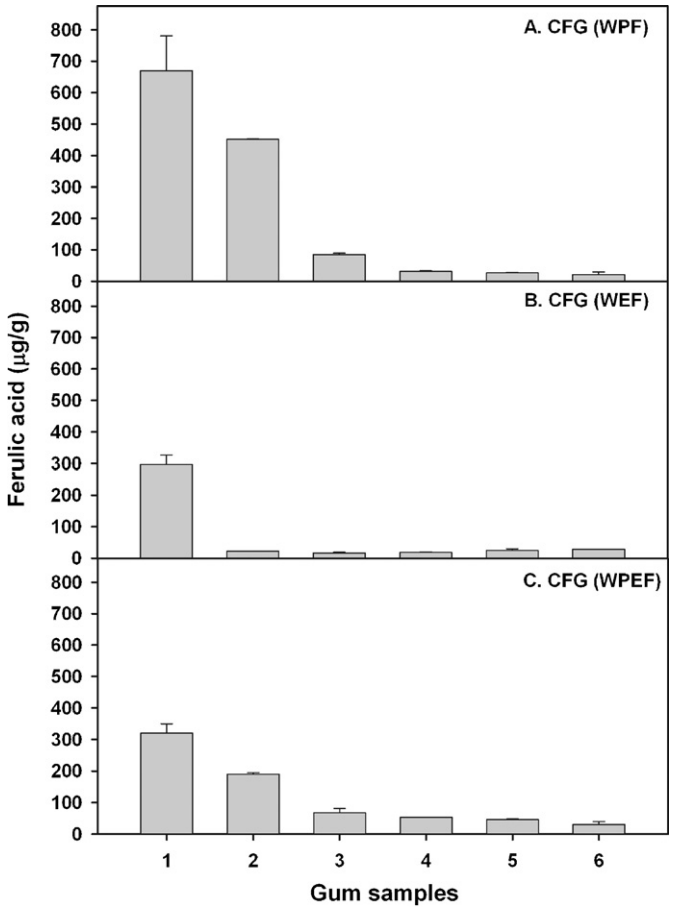

Fig. 3. Ferulic acid content of corn fiber gum isolated with 2, 4 or 8 meq alkali by heating at 100 °C. Alkali concentration and isolation time: (1) 2 meq, 15 min; (2) 2 meq, 1 h; (3) 4 meq, 15 min; (4) 4 meq, 1 h; (5) 8 meq, 15 min; (6) 8 meq, 1 h. Data are an average of three trials ± standard deviation.

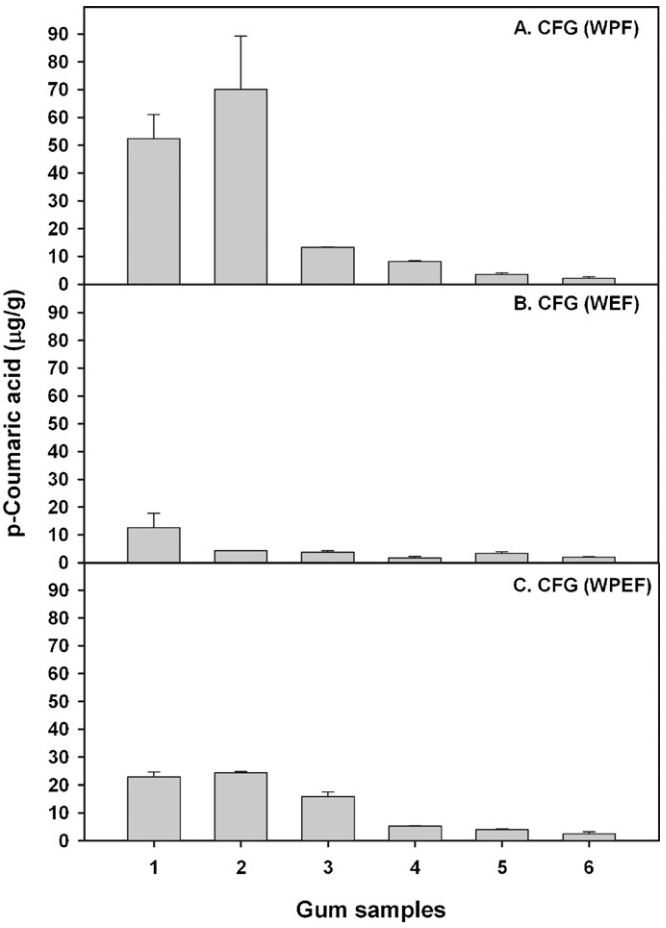

Fig. 4. p-Coumaric acid content of corn fiber gum isolated with 2, 4 or 8 meq alkali by heating at 100 °C. Alkali concentration and isolation time: (1) 2 meq, 15 min; (2) 2 meq, 1 h; (3) 4 meq, 15 min; (4) 4 meq, 1 h; (5) 8 meq, 15 min; (6) 8 meq, 1 h. Data are an average of three trials ± standard deviation.

concentration and a short heating time at 100 °C in presence of H_2O_2. These results totally agree with Carvajal-Millan et al. (2007), who reported that the weight percent of ferulic in corn arbinoxylan decreased from 1.6 to 0.6, 0.34, 0.22, 0.15 and 0.1 μg/mg as the incubation of corn bran in 0.5 M NaOH increased gradually from 2 h to 4, 8, 12, 16, and 24 h respectively. These results also support other investigators' findings that some phenolic acids are esterified to cell wall hemicelluloses in addition to their alkali stable ether linkage (Harvey, Hartly, Harris, & Curzon, 1986; Sun, Xiao, & Sun, 2001), which could be susceptible to high alkali concentration in the presence of H_2O_2, and longer treatment times. As we reported previously (Yadav, Moreau, et al., 2007), CFG isolated from WPF (pericarp originating fiber) contains higher amount of phenolic acids than WEF (endosperm originating fiber). This also agrees with Seitz (1989), who reported that most of the ferulate ester was present on the inner pericarp portion of the fiber and the endosperm portion of corn kernel contains lower levels of ferulic acids. The levels of phenolics in these CFGs isolated from different corn fiber sources are relatively low but they may provide limited nutraceutical, health promoting, or other functional benefits to foods and beverages to which they are added. They may also function as important antioxidants helping to maintain the quality of these food products. In addition to above mentioned applications, arabinoxylan from corn may have applications as a prebiotic and potential source of soluble dietary fiber thus increasing the value to the food or drink containing it.

4. Conclusions

The main findings of this study were the following:

1. The amount of protein and total phenolic acids in CFGs extracted from all three fiber sources by 2 meq alkali mixture/g fiber heating at 100 °C for 15 min is the highest in comparison to CFGs extracted with higher alkali concentration and longer heating time.
2. The protein content in CFG isolated from WEF is higher than the CFG isolated from WPF or WPEF.
3. The total phenolic acid content in CFG from coarse corn fiber (pericarp fiber) is comparatively higher than CFG from fine corn fiber (endosperm fiber) and the mixture of coarse and fine fiber.
4. The amount of lipid in CFG isolated from WPF with 2 meq alkali mixture/g fiber heating at 100 °C for 15 min is the highest in comparison to CFGs extracted with higher alkali concentration and longer heating time. But the lipid content in CFGs extracted from WEF and WPEF do not follow above trend and its difference under other isolation conditions is not so remarkable.

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