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ScienceDirect
Carbohydrate Polymers 71 (2008) 74–79
Carbohydrate Polymers
www.elsevier.com/locate/carbpol

Comparison of the effects of critical fluid and reflux-extracted techniques on cornstarch pasting properties

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Received 27 December 2006; received in revised form 3 May 2007; accepted 14 May 2007
Available online 21 May 2007

Abstract

Critical fluid extraction of native lipids from cornstarch using 75/25 (v/v) ethanol/water as the solvent removed over 99% of the native lipid. The percentage of native lipid extracted was altered by changing the solvent/starch ratio. The pasting properties and shear storage modulus of a defatted, critical fluid-extracted sample differed from those of a sample defatted by refluxing with 75/25 (v/v) *n*-propanol/water, although both samples contained only trace amounts of residual native lipid. The percent soluble starch in the lipid-extracted starches was higher than that of the control starch, which was expected since extraction of native lipids reduced the amount of lipid-complexed amylose within the starch granule and enabled more intragranular amylose to be leached out. This study describes a convenient, non-toxic process for extracting native lipids from cornstarch, and the altered pasting and gelling properties of these extracted starches could result in new end-use applications.
Published by Elsevier Ltd.

Keywords: Critical fluid extraction; Lipid removal; Starch; Pasting properties

1. Introduction

In recent years supercritical fluid extraction (SFE) methods in the field of starch chemistry have become more prevalent. These methods are convenient because easily controlled variables such as solvent flow time, temperature, and pressure are directly related to the efficiency of the extraction, giving the user a great deal of flexibility in the approaches available to customize the procedure to achieve a particular result. SFE methods also can be carried out with solvents of minimal toxicity (i.e. CO_2 and short chain alcohols). Hubbard, Downing, Ram, and Chung (2004) used supercritical CO_2 and CO_2/ethanol as an alternative to the traditional Soxhlet extraction of free lipids from wheat flour. Koxholt, Altieri, Marentis, and Trzasko (2003) used extraction with supercritical fluids to remove off-flavors, odors, and colors from starch. da Cruz Francisco and Sivik (2002) examined the gelatinization of cassava, potato, and wheat starches in supercritical CO_2. Braga, Moreschi, and Meireles (2006) studied supercritical fluid extraction of ginger and turmeric tuber starches in order to better understand molecular arrangement using X-ray diffraction. Although critical and supercritical

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0144-8617/$ - see front matter Published by Elsevier Ltd.
doi:10.1016/j.carbpol.2007.05.019

extraction methods have become more popular, none of the references listed above focused on removing the native lipids from starches and studying the pasting and gelling properties of the extracted starches.

Past research in our laboratory has explored the use of similar high-pressure methods to remove native lipids from cornstarch with different moisture contents using CO_2/ethanol and pure ethanol as solvent systems (Peterson, Eller, Fanta, Felker, & Shogren, 2007). Since the elevated temperatures and pressures used were not above the critical point for ethanol (243 °C and 926 psi), this procedure is referred to as critical fluid extraction (CFE) rather than SFE. Lipid was more efficiently extracted when pure ethanol was used and when the moisture content of the starch was 19% as opposed to 10%. However, a maximum of only 28% of the native lipids were extracted under these conditions. Although the native lipid content in cornstarch is less than 1% by weight, it plays a significant role in the pasting properties, since defatted starches display different pasting curves than their corresponding un-extracted controls.

Morrison and Coventry (1985) have shown that alcohol/water mixtures are efficient systems for removing native lipid from starch using conventional extraction techniques. Also, ethanol is non-toxic and would thus be suitable for extracting starches for food-related applications. In this study, a 75/25 (v/v) mixture of ethanol and water was used as the solvent for CFE of cornstarch, and different solvent/starch ratios were examined to see their effect on the pasting properties.

The goals of this research were (1) to increase the extent of lipid extraction over the 28% achieved previously (Peterson et al., 2007); (2) to compare the pasting properties of the CFE starches with both a control cornstarch (that was not extracted to remove the lipid) and a cornstarch sample that was conventionally extracted with refluxing 75/25 *n*-propanol/water; and (3) to measure the extent of granule swelling (i.e. swelling power) and the percentage of starch dissolved during pasting.

2. Materials and methods

2.1. Materials

Normal dent cornstarch with a moisture content of 9.2% (w/w) was obtained from A.E. Staley Mfg. Co., Decatur, IL. Percent moisture values were determined either by vacuum drying weighed starch samples at 100 °C or with an HFT 2000 moisture analyzer (Data Support Co. Inc., Encino, CA). All starch weights are given on a dry weight basis.

2.2. Lipid extraction

Lipid extraction using refluxing 75/25 (v/v) *n*-propanol/water was carried out, using 6 g of cornstarch and two consecutive 2 h extractions with 300 mL of refluxing 75/25 (v/v) *n*-propanol/water. After each extraction the mixture was vacuum filtered, and after the second extraction the solid was spread out in a Petri dish and allowed to air dry overnight. Critical fluid extractions were carried out in a custom-built SFE apparatus described previously (King, Johnson, & Friedrich, 1989). Two ISCO model 100DX syringe pumps (ISCO Corp., Lincoln, NE) were used to provide continuous solvent flow. Cornstarch (530 g) was added to a 1 L extraction cell (Thar Technologies Inc., Pittsburgh, PA). Samples were extracted at 1500 psi and 90 °C with 75/25 ethanol/water, at a constant flow rate of 5 mL/min. The solvent/starch ratio was thus controlled by changing the duration of the extraction; i.e. the solvent/starch ratio was directly proportional to the total flow time.

2.3. Starch pasting curves

Pasting curves were obtained using a TA AR2000 rheometer (TA Instruments, New Castle, DE) equipped with a starch pasting cell attachment. Starch concentrations were either 5% or 10% (w/w); and the appropriate dry weight was brought to 20 g total weight with DI water. The temperature and stirring conditions for all pasting profiles were as follows: An initial mixing step at 750 rpm was first applied for 30 s at 25 °C, and a linear temperature increase of 5 °C/min was then applied until the sample reached 95 °C. During this step and for the remainder of the pasting profile, the mixing head rotated at 100 rpm. The sample was held for 5 min at 95 °C, and the temperature was then decreased linearly at 5 °C/min to 25 °C.

For retrogradation experiments, upon conclusion of the temperature ramp back down to 25 °C, the rheometer was switched to an oscillatory time sweep test at 0.5% strain for 15 h.

2.4. Relative swelling power determination

To determine relative swelling power, a modified method based on that of Schoch (1964) was employed. In this method a single-point characterization at 95 °C was measured using the following procedure. A 5% solids starch suspension was prepared by diluting 1 g of starch and then adding sufficient water to bring the total weight to 20 g. This suspension was then placed into the AR2000 starch pasting cell, mixed for 30 s at 750 rpm and 25 °C, and then stirred at 200 rpm at 95 °C for 30 min. The stirring paddle and the contents of the starch pasting cell were then rinsed quantitatively into a tared beaker, and the sample was transferred to two centrifuge tubes and centrifuged for 15 min at 4700 rpm (2645*g*). The clear supernatant was decanted into an aluminum weighing pan, weighed and placed into a 50 °C oven overnight to dry, and then vacuum dried for at least 60 min. Each sample was run in triplicate. Percent soluble starch and swelling power were calculated as described by Schoch (1964).

2.5. Microscopy and birefringence loss

Pasted starch samples were diluted with a drop of water on a microscope slide and examined by phase contrast optics using a Zeiss Axioskop light microscope (Carl Zeiss, Inc., Thornwood, NY). For determination of birefringence loss during pasting, 1 g of starch was placed in a 150 mL beaker, and 100 mL distilled water (27 °C) was added. The dispersion was heated at 2 °C/min on a hot plate with magnetic stirring. Aliquots of 0.2 mL were removed at 2 °C intervals from 50 to 96 °C, cooled rapidly in a polystyrene microtiter plate in an ice bath, and examined with a Zeiss microscope equipped with crossed polarizing filters.

For scanning electron microscopy (SEM), an aqueous dispersion of particles (20 μL) was added to 20 mL of absolute ethanol and allowed to settle. Particles were washed with ethanol and then critical point dried onto aluminum stubs. Dried specimens were sputter coated with Au–Pd and examined with a JEOL 6400 V (JEOL USA, Inc., Peabody, MA) scanning electron microscope.

2.6. X-ray diffraction

X-ray powder diffraction analysis was performed with a Philips 1820 diffractometer operated at 40 kV, 30 mA with graphite filtered CuK_α radiation and a theta compensating slit. Data were acquired in 0.05° 2θ, 4 s steps. Samples were equilibrated at 23 °C, 50% r.h. for 3 days prior to analysis.

3. Results and discussion

The lipid extraction efficiency of CFE as a function of solvent/starch ratio is shown in Table 1. The amounts of extracted lipid were determined by esterification with HCl/methanol followed by a gas chromatographic procedure described previously (Peterson, Fanta, Adlof, & Felker, 2005). Extraction by refluxing with 75/25 *n*-propanol/water is also shown for comparison. Use of the *n*-propanol/water system to efficiently extract native lipid has been reported by Morrison and Coventry (1985), and the amount of native lipid that we extracted with this solvent system (7.52 mg/g of starch) was consistent with literature values reported for the total native lipid content of maize starch (Morrison, Milligan, & Azudin, 1984). Therefore, for the calculations of percent native lipid extracted by CFE in Table 1, it was assumed that lipid was completely removed by *n*-propanol/water extraction, and that the lipid content of un-extracted starch was 7.52 mg/g. For the CFE extractions, the solvent/starch ratio was controlled by holding the solvent flow rate constant and by increasing the amount of total solvent applied under pressure through the extraction cell. This procedure increased the time duration of the CFE treatment. When the solvent/starch ratio was increased from 3.74 to 11.9 mL/g starch, the percentage of native lipid extracted increased from 90.0 to 99.9, as compared with a maximum of 28% achieved in our previous study (Peterson et al., 2007).

Table 1
Lipid extraction efficiency as a function of solvent:starch ratio

Lipid extraction method	Solvent	Solvent: starch ratio (mL:g)	mg extracted lipid per g cornstarch	Percent native lipid extracted
CFE	75/25 ethanol/water	3.74	6.77	90.0
CFE	75/25 ethanol/water	4.10	7.20	95.7
CFE	75/25 ethanol/water	11.9	7.51	99.9
Reflux	75/25 *n*-propanol/water	100	7.52	100

X-ray diffraction patterns for each of the starch samples are shown in Fig. 1. No appreciable differences in the scattering patterns were observed, and all were A-type patterns typical of cornstarch. These results are similar to those obtained previously (Peterson et al., 2007). When examined for birefringence loss, similar behavior was observed for (1) the CFE sample with 99.9% lipid extracted, (2) the sample extracted with *n*-propanol/water, and (3) the control starch that still contained native lipid. For all three samples, birefringence was lost between 64 and 72 °C. Microscopy showed a broad distribution of granule sizes, but no trends in birefringence loss related to granule size were observed.

The pasting curves of the starch samples at 5% solids are shown in Fig. 2. When native lipids are extracted from cornstarch, the amylose that was previously complexed with lipid is free to either leach out of the granule more easily, or, to retrograde and crystallize within the granule and thus produce granules that are more rigid and less deformable. Both behaviors reduce granule swelling, which in turn reduce the overall viscosity. The pasting curves therefore depended upon the amount of native lipid remaining in

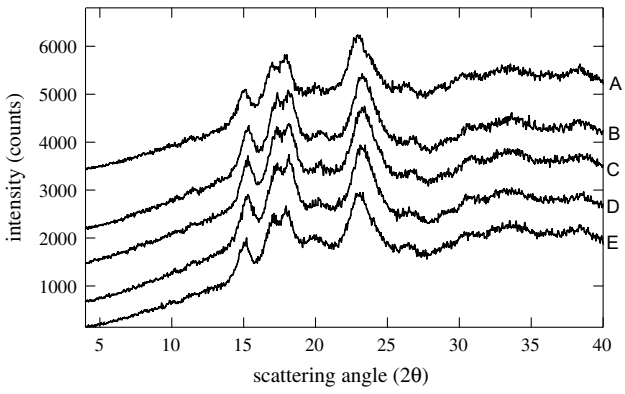

Fig. 1. X-ray diffraction patterns for cornstarch samples (traces have been vertically shifted for clarity): (A) extracted by refluxing with 75/25 *n*-propanol/water; (B) CFE with 11.9 mL/g solvent/starch; (C) CFE with 4.10 mL/g solvent/starch; (D) CFE with 3.74 mL/g solvent/starch; (E) starch control with no native lipid extracted.

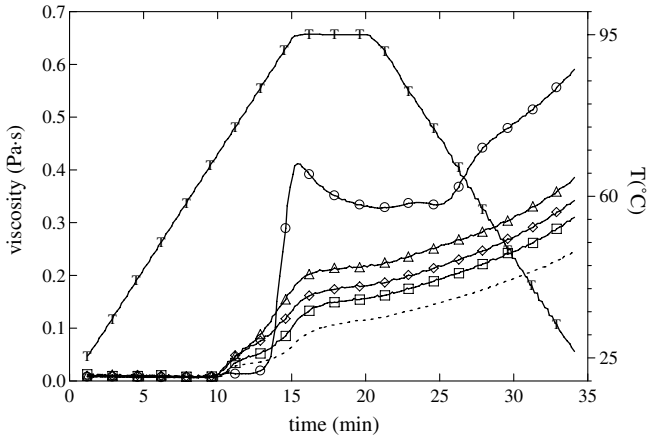

Fig. 2. Pasting profiles of cornstarch samples at 5% solids concentration. Symbol key: control starch = circles; CFE (3.74 mL solvent/g starch) = triangles; CFE (4.10 mL solvent/g starch) = diamonds; CFE (11.9 mL solvent/g starch) = squares; reflux-extracted starch = dotted line; temperature profile = '*T*' (right axis).

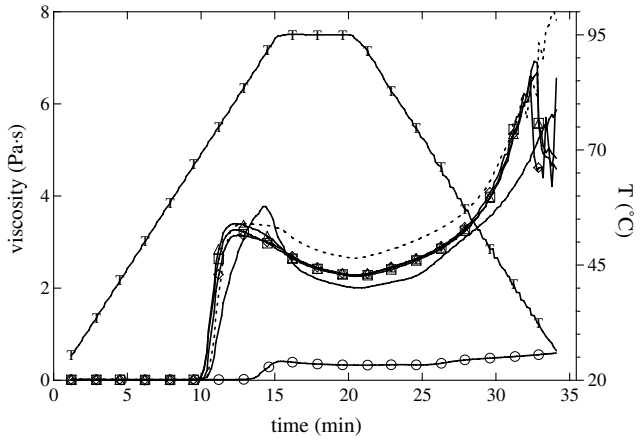

Fig. 3. Pasting profiles of cornstarch samples at 10% solids concentration. Symbol key: control starch = solid line; CFE (3.74 mL solvent/g starch) = triangles; CFE (4.10 mL solvent/g starch) = diamonds; CFE (11.9 mL solvent/g starch) = squares; reflux-extracted starch = dotted line; temperature profile = '*T*' (right axis). The circles represent the control starch at 5% (same trace as circles on Fig. 2) to illustrate the scale change in viscosity.

the sample after extraction, and the viscosity decreased with the amount of lipid remaining. Whereas the cornstarch control displayed typical pasting behavior (granule swelling, peak viscosity, shear-induced breakdown, and finally the onset of retrogradation), none of the CFE samples reached a sharp peak viscosity; the viscosities continued to slowly rise throughout the remainder of the pasting profile. The differences in viscosity between the CFE samples and the un-extracted control were greater than the differences observed in our earlier study (Peterson et al., 2007), due to the increased levels of lipid extraction; and the viscosities shown in Fig. 2 were closer to the reflux-extracted sample. However, the native lipid content of the starch was not the only factor that influenced pasting properties; since the reflux-extracted sample and the CFE sample with 99.9% of the native lipid removed had different pasting properties even though both samples contained essentially no residual lipid. Apparently, pasting properties are also affected by the method used for lipid extraction.

Pasting properties for the same series of samples were also determined at 10% starch solids (Fig. 3). At this higher concentration, there is less interstitial water and therefore more contact between individual swollen granules; increased viscosities of the pasted samples were therefore observed. The pasting curve for the control starch sample at 5% solids is shown on the same viscosity scale for comparison. The un-extracted starch control at 10% solids had a sharp peak viscosity that was higher than the CFE and reflux-extracted samples, which exhibited more plateau-like peak viscosities. The reflux-extracted sample showed the smallest drop in viscosity after the maximum plateau value, suggesting that the swollen granules were more rigid and resisted shear-induced breakdown more than the control starch.

The swelling power and the amount of soluble starch produced by swelling the starches in water at 95 °C were determined for the un-extracted control sample, the refluxed sample, and the CFE sample with 99.9% of the lipid removed, using the standard method of Schoch (1964). Values for swelling power and percent soluble starch are given in Table 2. Both CFE and reflux-extracted samples yielded more water-soluble starch than the un-extracted control starch, which suggests that native lipid was complexed with amylose and that extraction of lipid from the amylose helix increased its water solubility. Phase contrast micrographs of the un-dried, swollen starch granules obtained from the swelling power experiments are shown in Fig. 4. This figure shows that the two samples with lipid removed contained swollen granules that were, on average, slightly smaller in size, which correlated with the swelling power data from Table 2. Scanning electron micrographs of each of the dried lipid-extracted samples before pasting (Fig. 5) did not show any major differences in morphology compared to the un-extracted control starch.

Plots of the shear storage modulus (G') of (1) the un-extracted starch control, (2) the reflux-extracted sample, and (3) the CFE sample with 99.9% lipid removed, as a function of time (Fig. 6), show the increases in gel strengths observed when the pasted samples were allowed to retrograde for 15 h. As observed during pasting, the CFE sample and the reflux-extracted sample behaved differently, despite the fact that both samples contained only negligible amounts of residual native lipid. The CFE sample showed

Table 2
Percent soluble starch and swelling power of defatted cornstarch samples

Sample	Percent soluble starch	Swelling power
Control PFG	24 ± 4	27 ± 1
CFE 11.9 mL:g	33.7 ± 0.9	22.3 ± 0.5
Reflux-extracted	30.3 ± 0.1	19.2 ± 0.3

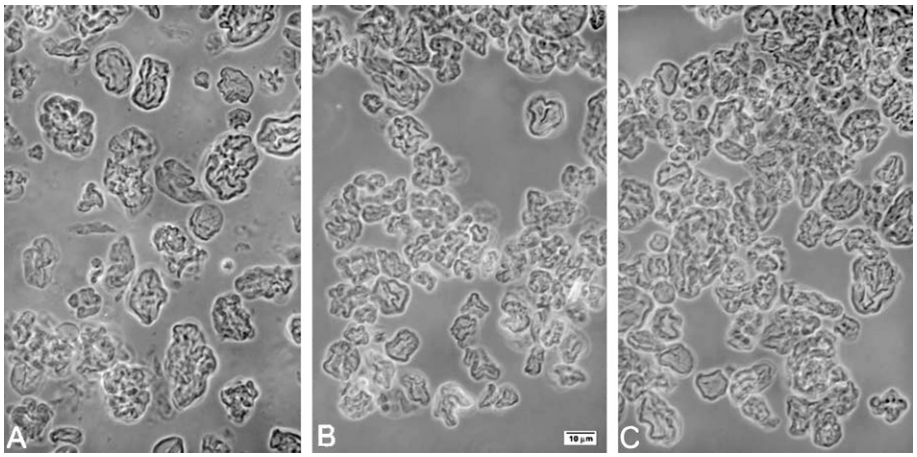

Fig. 4. Phase contrast microscopy images of pasted cornstarch samples following the swelling power heating and stirring protocol. (A) Control starch; (B) CFE (11.9 mL/g solvent/starch) starch; (C) reflux-extracted (75/25 *n*-propanol/water) starch.

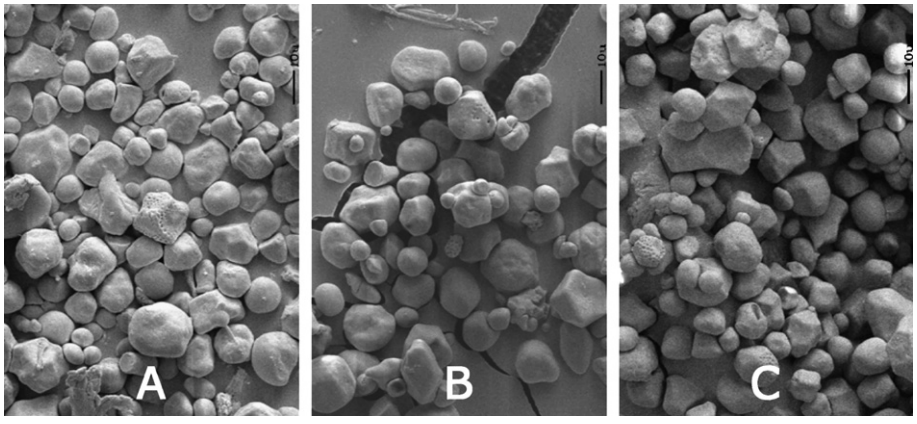

Fig. 5. SEM images of dried samples of (A) control starch; (B) CFE (11.9 mL/g solvent/starch) starch; (C) reflux-extracted (75/25 *n*-propanol/water) starch. The solid black bar in the upper-right section of each image corresponds to a distance of 10 μm.

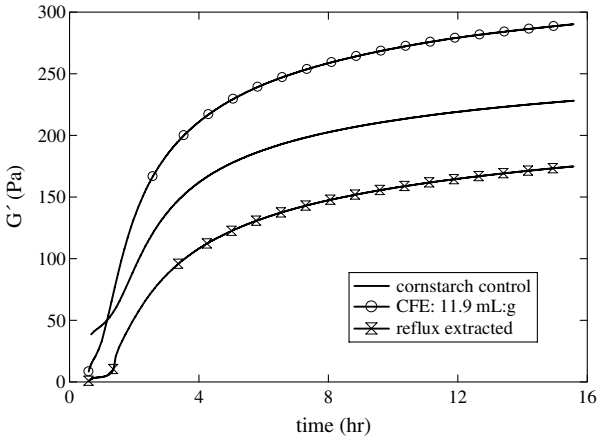

Fig. 6. G' vs. time for cornstarch samples at 5% solids concentration and 25 °C.

G' values that were higher than the cornstarch control throughout the entire 15 h cooling period; whereas the reflux-extracted sample showed G' values that were lower than the control. In our previous paper (Peterson et al., 2007), G' values for CFE starches with only 13%, 16%, and 28% of the native lipid extracted were also higher than the control. G' values depend to a large extent on the amount of amylose leached from the starch granules during pasting. Ellis and Ring (1985) observed that amylose gels have G' values that are strongly dependent on concentration (i.e. seventh power) above a critical concentration of 1.5% (w/w). The higher percentage of soluble starch for the CFE sample, as compared with the un-extracted starch control (Table 2), is therefore consistent with the higher G' values observed. However, G' must also be affected by other factors, for example, the presence of swollen granules and granule fragments with varying size and rigidity (Bagley & Christianson, 1982); since the G' values for reflux-extracted starch were lower than those of the control. It is not clear why there is such a significant difference in retrogradation behavior between these two samples that are both essentially defatted. Two other potential factors that may cause the difference in retrogradation behavior are (1) higher pressure during lipid extraction for the CFE sample; and (2) a higher solvent/starch ratio during lipid extraction for the reflux-extracted sample (see Table

1). Further research will be necessary to identify the factors responsible for the retrogradation behavior and determine their importance.

4. Conclusions

Critical fluid extractions with 75/25 ethanol/water were more effective in removing lipid from cornstarch than previous extractions that did not include water as a critical fluid solvent component (Peterson et al., 2007). The efficiency of lipid extraction increased with the solvent/starch ratio, and over 99% of the native lipid could be removed from normal dent cornstarch when a solvent/starch ratio of 11.9 mL/g was used. CFE starches were compared with starch that was conventionally defatted by extraction with 75/25 (v/v) *n*-propanol/water at reflux temperature. Neither of these extraction techniques produced discernable changes in the X-ray diffraction patterns, loss of birefringence, or the physical morphology of starch granules as observed by SEM.

Pasting curves of the lipid-extracted starches exhibited plateau-like peak viscosities, as opposed to the sharper peak viscosities observed with un-extracted cornstarch. The maximum viscosities and shapes of the pasting curves depended on the starch solids concentration, the amount of residual native lipid in the starch sample, and the method used for lipid extraction (CFE vs. 75/25 *n*-propanol/water). Plots of G' vs. time were obtained for the starch samples as they were allowed to retrograde for 15 h after pasting. Different results were obtained for the CFE and reflux-extracted samples, despite the fact that both samples were essentially defatted. The CFE sample showed G' values that were higher than the cornstarch control; whereas G' values lower than the control were observed for the reflux-extracted sample. This behavior does not appear to be controlled by soluble starch concentration in the dispersion. Other differentiating factors that occur during lipid extraction, such as pressure or the solvent/starch ratio may have a more significant role in contributing to the difference in retrogradation behavior, but further research will be necessary to determine whether this is the case or not.

The ability to remove over 99% of the native lipid from cornstarch by critical fluid extraction will provide manufacturers with a method for altering the pasting and gelling properties of cornstarch and thus extend the range of end-use applications. Since ethanol/water is a non-toxic solvent system, the extracted starches will be suitable for food applications.

Acknowledgements

The authors thank Elizabeth Krietemeyer for technical help with the X-ray diffraction spectra, and Arthur Thompson for carrying out the SEM microscopy experiments.

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