Carbohydrate Polymers 87 (2012) 2477–2481

Contents lists available at SciVerse ScienceDirect

Carbohydrate Polymers

journal homepage: www.elsevier.com/locate/carbpol

Pressurized solvent extraction of pure food grade starch☆

F.J. Eller[a,*], S.C. Peterson[b], D.J. Sessa[b]

[a] United States Department of Agriculture, Agricultural Research Service, National Center for Agricultural Utilization Research, Functional Foods Research Unit, 1815 North University Street, Peoria, IL 61604, USA
[b] United States Department of Agriculture, Agricultural Research Service, National Center for Agricultural Utilization Research, Plant Polymer Research Unit, 1815 North University Street, Peoria, IL 61604, USA

ARTICLE INFO

Article history:
Received 15 September 2011
Received in revised form 18 October 2011
Accepted 7 November 2011
Available online 15 November 2011

Keywords:
Cornstarch
Pressurized solvent extraction
Lipids
Zein
Pasting curve
Retrogradation

ABSTRACT

A commercial pressurized solvent extractor was used to remove lipid and non-lipid material from cornstarch using *n*-propanol/water and ethanol/water mixtures. Yields and chemical composition of the extract fractions were determined. Cornstarch samples were characterized using pasting properties and shear storage modulus measurements. The *n*-propanol/water extracted slightly higher amounts of both lipids and non-lipids. The lipid fractions contained mostly linoleic, palmitic and oleic free fatty acids. The non-lipid fraction contained mostly protein in the form of zein. The extracted starch had lower peak and setback viscosities than did the unextracted starch. The starch extracted with *n*-propanol/water had the lowest shear storage modulus values. Conversely, the samples extracted with ethanol/water had the highest shear storage modulus values. It is hypothesized that low amounts of zein present in conjunction with the starch is responsible for this observed effect.

Published by Elsevier Ltd.

1. Introduction

Cornstarch typically contains low amounts (ca. 1%) of native lipids with the free fatty acids linoleic, palmitic and oleic being the most abundant lipids present (Morrison, 1988; Morrison & Coventry, 1985). Although the relative amounts of these lipids are very low, the presence of these compounds can have a significant effect on the physical properties of the starch and subsequently its end-uses.The extraction of native cornstarch lipids using supercritical CO_2 (SC-CO_2), SC-CO_2 modified with ethanol (SC-CO_2/EtOH) and pressurized solvent extraction (PSE) using pure ethanol (EtOH) has been investigated (Peterson, Eller, Fanta, Felker, & Shogren, 2007) and compared to refluxing *n*-propanol/water (75/25, v/v) which removes essentially all of the lipids (Morrison & Coventry, 1985). Pure EtOH gave the highest yield, however, it only removed 28% of the lipids present in the cornstarch. However, the lipid yield using EtOH increased with the moisture content of the starch suggesting a benefit of water when extracting lipids using EtOH. In a subsequent study (Peterson, Eller, Fanta, Felker, & Shogren, 2008), using ethanol/water (75/25, v/v) in a lab-built pressurized solvent extractor, a solvent/starch ratio of ca. 12 was found to remove 99.9% of the native lipid from cornstarch.

The removal of the lipids by the refluxing 75% *n*-propanol/water was associated with a drastic decrease in the peak viscosity normally attributed to starch granule swelling and led to a significantly thinner slurry as indicated by the pasting profiles of the extracted cornstarch. Although the peak viscosity of starch extracted by EtOH was reduced relative to the control, it was not as drastic as seen for the 75% *n*-propanol/water extracted cornstarch sample. It appeared that the peak viscosity was proportional to the lipid content as has been reported previously (Goering, Jackson, & DeHaas, 1975; Kar, Jacquier, Morgan, Lyng, & McKenna, 2005; Sayar, Koksel, & Turhan, 2005; Takahashi & Seib, 1988; Vasanthan & Hoover, 1992). It is hypothesized that when lipids are removed from amylose–lipid complexes within the granule, the amylose may crystallize to a small degree and swelling would be reduced and at low granule concentrations, this leads to a lower paste viscosity. However, there are also cases where it appears that removal of native lipids either did not change the peak viscosity (Lorenz, 1976), or increased the peak viscosity and/or the setback region of the pasting curve (Biliaderis & Tonogai, 1991; Melvin, 1979).

Although amylose gels have shear storage modulus (G') values that are strongly dependent on concentration (i.e. seventh power) above a critical concentration of 1.5% (w/w) (Ellis and Ring (1985), the relationship between the amount of lipid extracted and the G' of the extracted starch is not clear (Peterson et al., 2007, 2008). The

☆ Mention of trade names or commercial products in this article is solely for the purpose of providing scientific information and does not imply recommendation or endorsement by the U.S. Department of Agriculture. USDA is an equal opportunity provider and employer.

* Corresponding author. Tel.: +1 309 681 6232; fax: +1 309 681 6685.
E-mail address: Fred.Eller@ARS.USDA.gov (F.J. Eller).

0144-8617/$ – see front matter. Published by Elsevier Ltd.
doi:10.1016/j.carbpol.2011.11.016

sample extracted by *n*-propanol/water (i.e., lowest lipid concentration) had the lowest G', while sample extracted by pure EtOH (i.e., with 28% of lipids removed) as well as the sample extracted with EtOH/water (75/25, v/v) (i.e., with 99.9% of lipids removed) both had higher G' than the unextracted control starch. It is unclear why G' increased for the EtOH extracted starch but it cannot be explained by the lipid content of the starch alone.

Both surface lipids and non-lipids (i.e., proteins) have been reported to influence the swelling properties of starch (Debet & Gidley, 2006). Various solvents have been shown to differentially extract lipids and non-lipids from starch. Nierle, El Baya, Kersting, and Meyer (1990) reported that ethanol extracted most of the wheat starch lipids present but only 25% of the proteins and described considerable changes in the rheological properties of the extracted wheat starch. Morrison and Coventry (1985) reported that propanol–water extracted substantial quantities of both lipids as well as prolamins (e.g., zein), while water-saturated *n*-butanol effectively lipids but only small amounts of prolamins. For this study, we choose to compare *n*-propanol–water and ethanol–water to differentially extract lipids and non-lipids from cornstarch as a continuation of our previous research.

The purpose of this study was to investigate the use of a commercial automated pressurized solvent extractor to remove lipids and non-lipids from cornstarch. The effectiveness of two solvents was compared and the composition of the lipid fraction and non-lipid fractions of the extracts were determined. The pasting and retrogradation properties of the extracted starch were also examined and compared to the extraction yields.

2. Materials and methods

2.1. Cornstarch

Pure food grade cornstarch was purchased from Tate and Lyle (lot # DW3C0406L; Decatur, IL). Percent moisture was determined to be 10.9% by drying overnight in a vacuum oven at 100 °C.

2.2. Accelerated solvent extractions

A Dionex Accelerated Solvent Extractor (ASE) 300 (Dionex Corp., Sunnyvale, CA) was used to extract the cornstarch using ethanol:water (75:25, vol:vol) and *n*-propanol:water (75:25, vol:vol). There were four extraction treatments: ethanol only (EtOH); *n*-propanol only (Prop); ethanol followed by *n*-propanol (EtOH/Prop); and *n*-propanol followed by ethanol (Prop/EtOH). The sample sizes were ca. 2 g and the extraction conditions were the same for both solvents as follows: 90 °C, 1500 psi, 7 min static, 8 cycles, 80% flush, 60 s purge.

Extracts were dried under a gentle stream of nitrogen to a constant weight directly in the ASE collection vials. All yield data (g extract/100 g cornstarch) were based on the weight of the undried cornstarch. There were three replications of each extraction method.

2.3. Separation of extract into lipid and non-lipid fractions

The dried residues were extracted with *n*-hexane to separate lipid material from non-lipid material. The dried extracts were covered with 10 mL of HPLC grade *n*-hexane (Fisher Scientific, Fair Lawn, NJ) and subsequently placed in a Branson model 3510 ultrasonic cleaner (Danbury, CT) for 5 min to thoroughly disperse the sample. The dispersed sample in *n*-hexane was centrifuged at 2550 rpm for 10 min (IEC Clinical Centrifuge, International Equipment Company, Needham, MA) and the supernatant *n*-hexane removed. This procedure was repeated three times for each sample and the combined *n*-hexane extracts were dried under a gentle stream of nitrogen and weighed. The insoluble non-lipid residue pellet was dried under nitrogen and weighed.

2.4. Supercritical fluid chromatographic analyses of extracts

Supercritical fluid chromatography (SFC) was used to determine the types of lipids present in the lipid extract fractions. The SFC analyses were conducted with a Series 4000 SFC (Selerity Technologies, Inc., Salt Lake City, UT, USA) equipped with a flame ionization detector (FID) held at 350 °C. SFC/supercritical fluid extraction-grade carbon dioxide (Airgas Inc., Radnor, PA, USA) was used as the carrier fluid. A SB-Methyl-100 capillary column (10 m by 50 μm i.d., 0.25 μm film thickness) (Selerity Technologies, Inc., Salt Lake City, UT, USA) was used with a program of: 100 °C isothermal, 10.1 MPa hold for 5 min, and a ramp of 1.5 MPa/min to 31.4 MPa. A solution containing ca. 5 mg/mL *n*-hexane was injected into the SFC (500 nL loop) and the relative amounts of components present were determined from the FID area percentages. A single SFC analysis was performed on each replication. Palmitic and stearic free fatty acid analytical standards were obtained from Aldrich Chemical Co. (Milwaukee, WI) and Matheson Co. Inc. (Norwood, OH), respectively.

2.5. Transesterification to fatty acid methyl esters

The extracted lipids were transesterified to fatty acid methyl esters (FAMEs) as described by House, Larson, Johnson, DeVries, and Martin (1994). The fat residue was dissolved in 1 mL of toluene and placed in a 12-mL screwcap vial with 1 mL of 7% BF_3 in methanol. The vial was then sealed and heated to 100 °C for 45 min with gentle mixing every 10 min. The vial was removed from the oven and cooled to room temperature. A 5-mL aliquot of distilled water, 1 mL of *n*-hexane, and 1 g of Na_2SO_4 was added to the vial and mixed vigorously. Two layers were allowed to form (centrifuged to speed separation), and the top layer removed and dried over ca. 1 g of anhydrous Na_2SO_4 in a separate vial. Boron trifluoride (14% BF_3 in methanol) was purchased from Alltech, Inc. (Deerfield, IL).

2.6. Gas chromatographic analysis of FAMEs

FAMEs were analyzed by gas chromatography (GC) according to the method of House et al. (1994). The FAMEs were analyzed by split injection (200:1 split ratio) onto a Hewlett-Packard Model 5890 series II GC equipped with a flame ionization detector. The column used for analyzing the FAME derivatives was a SP-2340 (60 m, 0.25-mm diameter, 0.20-μm film thickness) (Supelco Inc., Bellefonte, PA), and the carrier gas was He, utilizing a linear flow velocity of 18 cm/s through the column. The temperature programmed was 100 °C for 5 min, 3 °C/min–190 °C, 1 °C/min–200 °C and held for 15 min, 50 °C/min–250 °C and held for 1 min. The injector and detector temperatures were 235 °C and 250 °C, respectively. Injections were made using a Hewlett-Packard 7673 auto injector and the sample volume injected was 1 μL. The chromatographic data were acquired using ChemStation software. FAMEs were identified by comparison to FAMEs in GLC-85 FAME standard mix (NuChek Prep, Inc., Elysian, MN).

2.7. Elemental analyses

Elemental analyses of the non-lipid fractions were performed on a Perkin Elmer 2400 Series II Dumas-type elemental analyzer (Waltham, MA). Samples were dried overnight in a vacuum oven at 105 °C and 68 cm Hg. Approximately 2–4 mg of material was used for each measurement. Calibration was performed using an acetanilide standard (Perkin Elmer PN 0240-1121) (C 1.09%; H 6.71%; N 10.36%). There were three replications of each treatment.

2.8. Gel electrophoresis

Gel electrophoresis of the non-lipid fractions were performed using a 1.5 mm × 10 well NuPaGE 4–12% Bis-Tris Gel and MES-SDS running buffer (Invitrogen, Carlsbad, CA). The sample buffer contained 0.055 M Tris (pH 6.8); 2% SDS; 7% glycerol; 4.38% 2-mercaptoethanol and 5 M urea. Samples (ca. 2.5 mg/250 μL sample buffer) were held in boiling water bath for ca. 5 min then 10 μL of each sample and standards were loaded into the sample wells.

There were 10 lanes including 3 lanes of molecular weight standards; 3 lanes containing the 3 replications of the *n*-propanol/water extract; 3 lanes containing the 3 replications of the ethanol/water extract; and one lane containing Freeman zein (Freeman Industries LLC, Tuckahoe, NY). The molecular weight standards (BioRad broad range) included: Myosin (200,000); β-galactosidase (116,250); Phosphorylase B (97,400); Serum albumin (66,200); Ovalbumin (45,000); Carbonic anhydrase (31,000); Trypsin inhibitor (21,500); Lysozyme (14,400); and Aprotinin (6500). The gel was run at a maximum of 120 ma, 200 V for ca. 1 h.

2.9. Starch pasting curves and shear storage modulus (G′)

Pasting curves were obtained using a TA AR2000 rheometer (TA Instruments, New Castle, DE) utilizing a starch pasting cell attachment. Samples were prepared at 5% starch solids; approximately 1 g (dry weight) brought to 20 g total weight with deionized water. First, an initial mixing step at 750 rpm was applied for 30 s at 25 °C. Then, a linear temperature increase of 5 °C/min was 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 at 95 °C for 5 min and then the temperature was decreased linearly at 5 °C/min–25 °C.

For retrogradation studies, once 25 °C was reached, the sample was immediately subjected to an oscillatory time sweep test and oscillated at 0.5% strain for a minimum of 15 h.

2.10. Statistical analyses

Statistical analyses were performed using Statistix 7 software (Analytical Software, Tallahassee, FL, USA). Analyses of variance (ANOVA) were conducted on percentage data and main effects were tested using *F*-tests with means were compared using Least Significant Difference (LSD). Correlation coefficients were determined using Pearson's correlation.

3. Results and discussion

3.1. Accelerated solvent extraction yields

The total amounts of material extracted from the cornstarch by the various solvents are shown in Fig. 1. The ANOVA indicated that there were highly significant effects of solvent on yield ($F_{5,42}$ = 2447, P = 0.0000). The *n*-propanol/water gave a slightly higher yield than ethanol/water (i.e., 0.81% versus 0.72%, respectively). The *n*-propanol/water after ethanol/water also gave a slightly higher yield than ethanol/water after *n*-propanol/water (i.e., 0.13% versus 0.06%, respectively). Thus, *n*-propanol/water is a slightly better solvent for extracting materials from cornstarch than ethanol/water. The total yield for ethanol/water followed by *n*-propanol/water was statistically equivalent to that for *n*-propanol/water followed by ethanol/water (i.e., 0.88% versus 0.84%, respectively), indicating the order of the two solvents does not affect the total yield.

The effects of extraction solvent on lipid and non-lipid fraction yields from cornstarch are shown in Table 1. The ANOVAs indicated that there were highly significant effects of solvent on both lipid yield as well as non-lipid yield ($F_{5,42}$ = 2511, P = 0.0000 and $F_{5,42}$ = 792, P = 0.0000, respectively). The *n*-propanol/water gave a slightly higher yield of lipid than did ethanol/water (i.e., 0.50% versus 0.45%, respectively). The *n*-propanol/water after ethanol/water also gave a slightly higher lipid yield than ethanol/water after *n*-propanol/water (i.e., 0.07% versus 0.02%, respectively). The order of the solvents did not affect the total amount of lipid extracted, both ethanol/water followed by *n*-propanol/water and *n*-propanol/water followed by ethanol/water gave identical lipid yields (i.e., 0.52%). The cornstarch used in this study is reported to contain 0.47% fat, so both the *n*-propanol/water and the ethanol/water extractions were quite effective at extracting the lipids from this material.

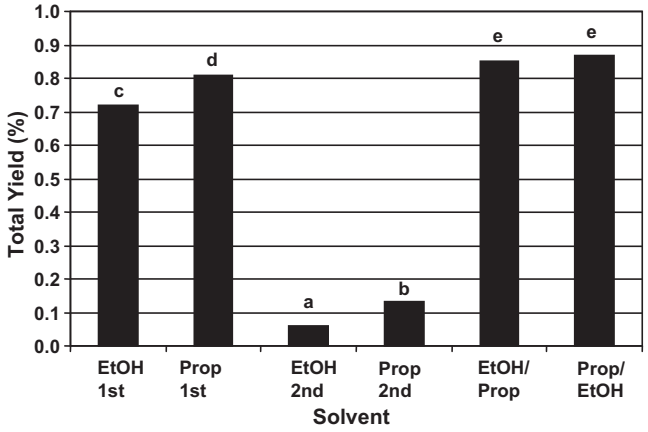

Fig. 1. Effect of solvent on total mass extracted from cornstarch.

The non-lipid yields followed the same pattern as the total yield and lipid yields with *n*-propanol/water extracting more than ethanol/water but the overall yield for the two solvents being equivalent. There was a highly significant correlation (i.e., 0.9887, n = 48, P = 0.0000) between lipid yield and non-lipid yield indicating that these two fractions have very similar solubilities in *n*-propanol/water and ethanol/water.

3.2. Supercritical fluid chromatographic analyses of extracts

The SFC analyses revealed that the lipid fraction extracts were comprised of mainly free fatty acids, of 16 and 18 carbon chain lengths. These free fatty acids constituted over 90% of the lipid material in the *n*-hexane extracts. The ANOVAs indicated that there were highly significant effects of solvent on both the 16 and 18 carbon chain length percentages ($F_{3,32}$ = 769, P = 0.0000 and $F_{3,32}$ = 1340, P = 0.0000, respectively). There was also a highly significant inverse correlation (i.e., −0.9905, n = 36, P = 0.0000) between the percentage of the 16 and 18 carbon chain lengths. There was always a higher percentage of 18 carbon fatty acids in all extracts, however, it was most pronounced in the first extractions. Ethanol/water extracted a slightly higher percentage of 18 carbon fatty acid than did *n*-propanol/water while *n*-propanol/water extracted a slightly higher percentage of 16 carbon fatty acid. The

Table 1
Effect of extraction solvent on fraction yields from cornstarch.

Extraction solvent	Lipid yield	Non-lipid yield
Ethanol first	0.45 c	0.29 c
Propanol first	0.50 d	0.30 c
Ethanol second	0.02 a	0.04 a
Propanol second	0.07 b	0.06 b
Ethanol/propanol	0.52 e	0.35 d
Propanol/ethanol	0.52 e	0.35 d

Means (n = 3) within a column without letters in common differ significantly by LSD (P = 0.05).

Table 2
Effect of extraction solvent on fatty acid composition of lipid fraction.

Extraction solvent	Fatty acid percentage				
	C16:0	C18:0	C18:1	C18:2	C18:3
Ethanol first	27.7 a	2.7 a	11.8 a	53.5 d	4.0 c
Propanol first	30.4 b	3.2 b	12.5 a	50.1 c	3.5 b
Ethanol second	46.3 c	9.1 d	22.2 c	22.3 b	0.0 a
Propanol second	52.8 d	7.3 c	20.2 b	19.7 a	0.0 a

Means ($n = 3$) within a column without letters in common differ significantly by LSD ($P = 0.05$).

percentages of 18 carbon fatty acid in the extracts of the second solvent were significantly less than those of the first solvents.

3.3. Fatty acid compositions of extracts

The results of the GC-FAME analyses of the lipid fractions of the cornstarch extracts are shown in Table 2. The GC analyses revealed that there were highly significant solvent effects on the five major fatty acids present in the extracts. The *F*-values (*P*-values) for the fatty acids were: palmitic $F_{3,8} = 368.4$ (0.0000), stearic $F_{3,8} = 210.7$ ($P = 0.0000$), oleic $F_{3,8} = 279.5$ ($P = 0.0000$), linoleic $F_{3,8} = 272.2$ (0.0000) and linolenic acid $F_{3,8} = 336.9$ (0.0000). There were also highly significant inverse correlations between the percentage of oleic acid and palmitic acid (i.e., −0.9869, $n = 12$, $P = 0.0000$) and oleic acid and linoleic acid (i.e., −0.9766, $n = 12$, $P = 0.0000$). Palmitic acid and oleic acid were positively correlated (i.e., 0.9297, $n = 12$, $P = 0.0000$).

3.4. Elemental analysis

The results of the elemental analyses of the non-lipid extracts are shown in Table 3. The ANOVAs indicated that there was no significant effect of solvent on either percent carbon or percent hydrogen ($F_{1,4} = 0.70$, $P = 0.45$; $F_{1,4} = 6.98$, $P = 0.0575$, respectively). However, there was a significant effect of solvent on percentage nitrogen ($F_{1,4} = 8.4$, $P = 0.0445$). Both the ethanol/water extract and the *n*-propanol/water extract contained significant amounts of nitrogen with the ethanol/water extract containing significantly more than the *n*-propanol/water extract. The protein content of these samples was estimated by multiplying the %*N* values by 6.25 and gave estimates of 59.4% and 54.4% for the ethanol/water and *n*-propanol/water extracts, respectively. The non-lipid fractions of both extracts apparently contain high percentages of proteins. The cornstarch used in this study is reported to contain 0.26% protein, so both the *n*-propanol/water and the ethanol/water extractions were quite effective at extracting the lipids from this material, with our values slightly higher than the reported values.

3.5. Gel electrophoresis

The gel of the molecular weight standards, 3 replications of the *n*-propanol/water extracts, 3 replications of the ethanol/water extracts; and the Freeman zein standard are shown in Fig. 2. The three replications of the *n*-propanol/water extracts were identical to each other as well as to the three replications of the ethanol/water extracts. In addition, the extracts of both solvents were nearly a perfect match with the Freeman zein standard. It is apparent that both non-lipid fractions of the *n*-propanol/water extracts and the ethanol/water extract contained zein. Hojilla-Evangelista, Myers, and Johnson (1992) identified zein as the protein extracted from flaked, defatted, whole corn during sequential extraction processing.

Table 3
Effect of extraction solvent on elemental composition of non-lipid extract.

Extraction solvent	Element		
	C	H	N
Ethanol first	53.9 a	8.5 b	9.5 b
Propanol first	54.3 a	8.7 a	8.7 a

Means ($n = 3$) within a column without letters in common differ significantly by LSD ($P = 0.05$).

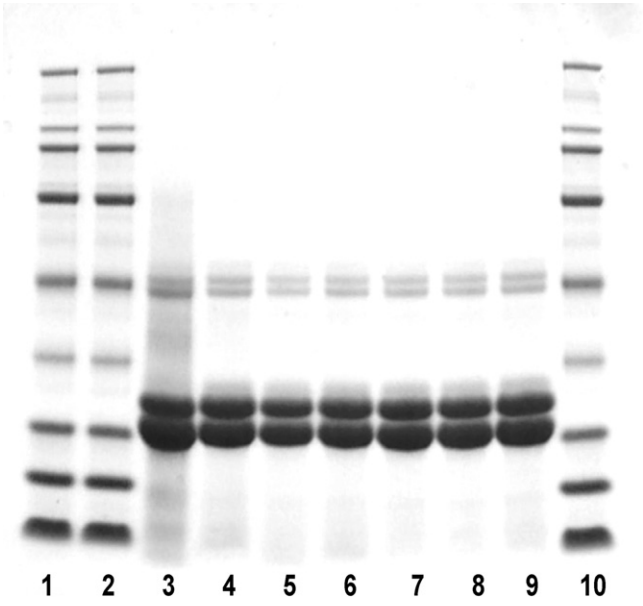

Fig. 2. Gel of molecular weight standards and extracts from cornstarch. Lanes 1, 2 10: molecular weight standards; lane 3: Freeman zein; lanes 4–6: propanol/water extract; lanes 7–9: ethanol/water extract.

3.6. Starch pasting curves and shear storage modulus (G′)

The starch pasting curves for extracted and un-extracted cornstarch are shown in Fig. 3. The pasting curve for the unextracted control cornstarch was essentially identical to previous reports (Peterson et al., 2007, 2008). As expected, the pasting profile for the *n*-propanol/water extracted cornstarch was also essentially identical to that of cornstarch extracted with refluxing *n*-propanol/water only as previously described (Peterson et al.,

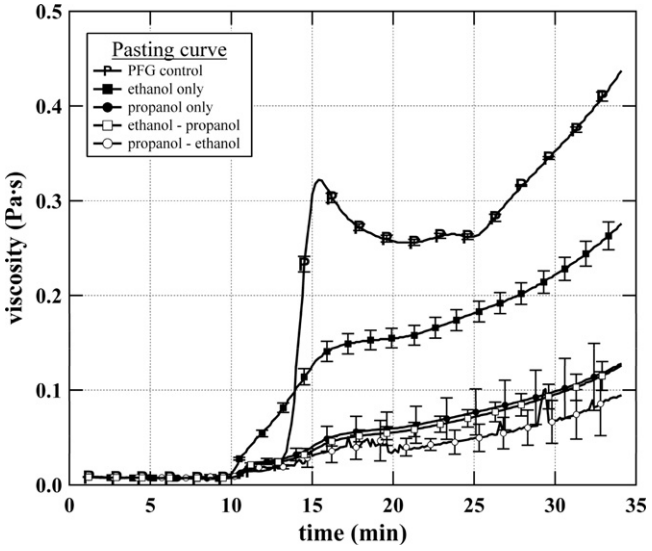

Fig. 3. Pasting curves for extracted and un-extracted cornstarch.

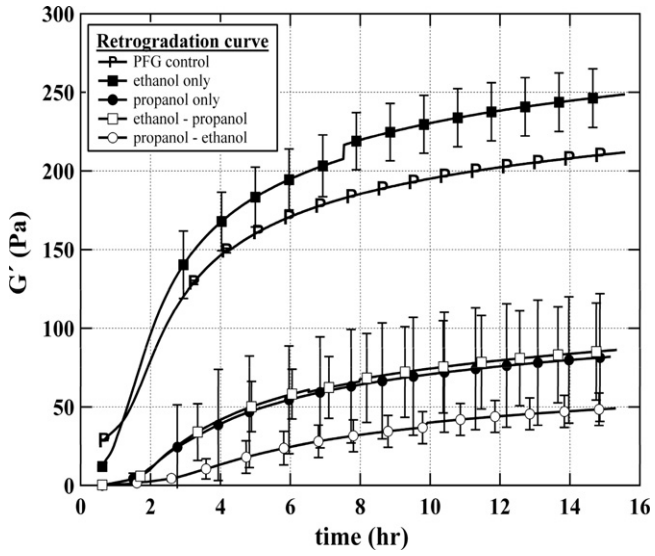

Fig. 4. G' versus time for extracted and un-extracted cornstarch.

2007, 2008). The pasting profiles of ethanol/water only, ethanol/*n*-propanol, and *n*-propanol/ethanol extracted cornstarch were very similar to the *n*-propanol/water only sample. However, the cornstarch extracted with ethanol/water only was intermediate between that seen for the unextracted control and was quite different from the other treatments which included *n*-propanol/water. However, the ethanol/water only extracted starch gave results that were similar to what has been described for this treatment (Peterson et al., 2007, 2008).

The plots of the shear storage modulus (G') for extracted and un-extracted cornstarch samples are shown in Fig. 4. In a similar fashion, all of the extractions which included *n*-propanol/water are grouped together and show a large decrease in G' relative to the unextracted control. Interestingly, the G' for the ethanol/water only extracted starch were actually higher than that seen for the unextracted control rather than being intermediate between the *n*-propanol/water extracted samples and the unextracted control sample. This may be a result of the slightly higher extraction of lipids and non-lipids (i.e., zein) by the *n*-propanol/water. The small amounts of these zein proteins left by the ethanol–water may be enough to cause a slight increase in G' after cooling by their interactions with one another on the surface of the cornstarch granules.

4. Conclusions

This study describes the use of a commercial pressurized solvent extractor as a convenient method to extract both lipid and non-lipid materials such as free fatty acids and zein from cornstarch. Although the extracted cornstarch may have only very low amounts of residual material such as lipids or zein, the presence of these materials can have a significant effect on the physical properties of the starch and subsequently its end-uses.

Acknowledgments

The authors wish to thank Jeffrey Teel, Andrew Thomas, Gary Grose, Mardell Schaer and Jeanette Little for their technical assistance.

References

Biliaderis, C. G., & Tonogai, J. R. (1991). Influence of lipids on the thermal & mechanical properties of concentrated starch gels. *Journal of Agricultural and Food Chemistry*, *39*, 833–840.
Debet, M. R., & Gidley, M. J. (2006). Three classes of starch granule swelling: Influence of surface proteins and lipids. *Carbohydrate Polymers*, *64*, 452–465.
Ellis, H. S., & Ring, S. G. (1985). A study of some factors influencing amylose gelation. *Carbohydrate Polymers*, *5*, 201–213.
Goering, K. J., Jackson, L. L., & DeHaas, B. W. (1975). Effect of some nonstarch components in corn and barley starch granules on the viscosity of heated starch–water suspensions. *Cereal Chemistry*, *52*, 493–500.
Hojilla-Evangelista, M. P., Myers, D. J., & Johnson, L. A. (1992). Characterization of protein extracted from flaked, defatted, whole corn by the sequential extraction process. *Journal of the American Oil Chemists' Society*, *69*, 199–204.
House, S. D., Larson, P. A., Johnson, R. R., DeVries, J. W., & Martin, D. L. (1994). Gas chromatographic determination of total fat extracted from food samples using hydrolysis in the presence of antioxidant. *Journal Association Official Analytical Chemists*, *77*, 960–965.
Kar, A., Jacquier, J. C., Morgan, D. J., Lyng, J. G., & McKenna, B. M. (2005). Influence of lipid extraction process on the rheological characteristics, swelling power, and granule size of rice starches in excess water. *Journal of Agricultural and Food Chemistry*, *53*, 8259–8264.
Lorenz, K. (1976). Physicochemical properties of lipid-free cereal starches. *Journal of Food Science*, *41*, 1357–1359.
Melvin, M. A. (1979). The effect of extractable lipid on the viscosity characteristics of corn and wheat starches. *Journal of the Science of Food and Agriculture*, *30*, 731–738.
Morrison, W. R. (1988). Lipids in cereal starches: A review. *Journal of Cereal Science*, *8*, 1–15.
Morrison, W. R., & Coventry, A. M. (1985). Extraction of lipids from cereal starches with hot aqueous alcohols. *Starch*, *37*, 83–87.
Nierle, W., El Baya, A. W., Kersting, H. J., & Meyer, D. (1990). Lipids and rheological properties of starch. *Starch*, *42*, 268–270.
Peterson, S. C., Eller, F. J., Fanta, G. F., Felker, F. C., & Shogren, R. L. (2007). Effects of critical fluid lipid extraction on the gelatinization and retrogradation of normal dent cornstarch. *Carbohydrate Polymers*, *67*, 390–397.
Peterson, S. C., Eller, F. J., Fanta, G. F., Felker, F. C., & Shogren, R. L. (2008). Comparison of the effects of critical fluid and reflux-extracted techniques on cornstarch pasting properties. *Carbohydrate Polymers*, *71*, 74–79.
Sayar, S., Koksel, H., & Turhan, M. (2005). The effects of protein-rich fraction and defatting on pasting behavior of chickpea starch. *Starch*, *57*, 599–604.
Takahashi, S., & Seib, P. A. (1988). Paste and gel properties of prime corn and wheat starches with and without native lipids. *Cereal Chemistry*, *65*, 474–483.
Vasanthan, T., & Hoover, R. (1992). Effect of defatting on starch structure and physicochemical properties. *Food Chemistry*, *45*, 337–347.