NOTE

Water Solubility and Macromolecular Properties of Corn Meal Extrudates as Affected by Epichlorohydrin[1]

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ABSTRACT

Degermed corn meal adjusted to 18% moisture content (db) with epichlorohydrin (ECH) content at 0, 0.5, 1, or 2% (w/w) were extruded with a twin-screw laboratory extruder at a screw speed of 140 rpm. Compression and metering barrel zones were set at 100, 120, or 140°C. Water solubility (WS) of ground extrudates ranged from 7.6 ± 1.1% to 14.3 ± 1.3%. ECH content had a significant ($P < 0.01$) negative effect on WS, while barrel temperature and the interaction between barrel temperature and ECH content were not significant ($P > 0.05$). Presumably, ECH reduced WS of extrudates through cross-linking between hydroxyl groups on starch and protein molecules. Gel-permeation chromatography patterns for both 100 and 140°C barrel temperatures showed that high molecular weight carbohydrates in the extrudates decreased with increasing ECH content without a simultaneous increase in low molecular weight carbohydrates. This suggested that the decrease in high molecular weight fractions was due to insolubilization by cross-linking rather than degradation. SDS-PAGE revealed that two protein bands of ≈29 and 17.5 kDa disappeared, and a new band appeared at 45 kDa with increasing ECH content. This indicated that, most likely, ECH reacted with protein in addition to reacting with starch. However, glycoprotein and starch-protein complexes were not identified with electrophoresis.

In recent years, environmental concerns such as ozone layer destruction and limited space for landfills have been discussed. Petroleum-based materials such as loose-fill packagings are closely related to these problems because they are nondegradable and are made using chrolofluorocarbons (CFC) as blowing agents. CFC are harmful to the ozone layer (Molina and Rowland 1974). Efforts have been made to replace petroleum-based materials with renewable, biodegradable ones in some situations.

Natural macromolecules, such as starch and protein, can be expanded using high-temperature, short-time extrusion cooking processes without blowing agents. Several researchers have tried to improve properties of starch-based extrudates for development of bioplastics such as loose-fill packaging foams (Altieri and Lacourse 1992; Bhatnagar and Hanna 1995, 1996; Lin et al 1995; Takamine et al 1995). The limiting characteristic of these materials is low water resistance. Improving the water resistance of starch-based extruded foams will increase their functionality as loose-fill packagings.

Zein, the prolamin and main fraction of corn protein, is relatively hydrophobic and has been used for preparing degradable edible films (Gennadios and Weller 1990, Park et al 1994, Yamada et al 1995, Herald et al 1996) and compression moldings (Jane et al 1993, Lim and Jane 1993, Spence et al 1995). Compression-molded plastic-like articles from corn starch and zein (5:1 weight ratio) had improved water resistance when compared to control starch moldings (Jane et al 1993, Lim and Jane 1993). Further significant improvements in water resistance of starch-zein moldings were realized by treatment with the protein cross-linking agents formaldehyde and glutaraldehyde (Jane et al 1993, Lim and Jane 1993). Also, dialdehyde starch-zein (3:1) moldings had significantly lower water absorption than normal starch-zein (3:1) moldings, suggesting that cross-linking occurred between dialdehyde starch and zein (Spence et al 1995).

Epichlorohydrin (ECH) is a cross-linking agent promoting covalent bonding between adjacent hydroxyl groups (–OH) in starch (Roberts 1964). Wheat, corn, and potato starches have effectively been cross-linked with small amounts of ECH (Gough 1967, Kartha and Srivastava 1985, Jane et al 1992). Reportedly, cross-linking with ECH significantly decreased starch solubility in water (Kartha and Srivastava 1985). Therefore, any ECH-induced cross-linking of starch would be expected to increase water resistance of extruded starch-based materials. We undertook this study to quantify the effect of ECH on water solubility of corn meal extrudates. Molecular changes of starch and protein in the extrudates also were studied.

MATERIALS AND METHODS

Sample Preparation

Yellow corn meal (Buckeye Pure Gold Corn Meal) was obtained from the Quaker Oats Co. (Chicago, IL). The moisture content of the corn meal was determined by drying samples in a forced convection oven (105°C for 24 hr), cooling them in a desiccator over anhydrous calcium sulfate (0% rh), and weighing them to the nearest ±0.01 g. Corn meal samples were then mixed with distilled water in a Hobart mixer (model C-100) to adjust the moisture content to 18% (db). Subsequently, ECH was mixed in at concentrations of 0, 0.5, 1.0, or 2.0% (dwb). All samples were stored in plastic containers at ambient temperature overnight before extrusion.

Extrusion

A corotating twin-screw laboratory extruder (model CTSE-V, C.W. Brabender, South Hackensack, NJ) was used (conical intermeshing screws; 36.5-cm barrel length; decreasing screw dia. from 4.3 to 2.8 cm; 3:1 compression ratio; 3-mm dia. short die nozzle; 15-mm die land length). The feed barrel zone was held at 70°C while the compression and metering barrel zones and the die

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were set at 100, 120, or 140°C. Screw speed was set at 140 rpm. Samples were hand-fed keeping the extruder's feed opening full at all times ("choke-fed") to achieve "full feeding". Addition of ECH did not appreciably affect operating torque and pressure. The cylindrical ropelike extrudates (expansion ratios of 9.6–18.7) were collected on aluminum foil-covered plastic trays and dried at 50°C in a walk-in dryer overnight. A portion of dried extrudates was ground in a Powdertec 3090 micro-mill (Tecater Inc., Silver Spring, MD) to pass through a 60-mesh sieve.

Water Solubility

A 0.5-g sample of the ground extrudate was placed in a flask. Then 50 mL of a 0.02% (w/v) aqueous sodium azide (to prevent microbial growth) solution were added. The flasks were incubated at 30°C for 18 hr under mechanical shaking in a constant temperature waterbath. At the end of the incubation period, the contents were centrifuged at 5,000 × *g* for 30 min. The supernatant was filtered through Whatman 42 filter paper, and carbohydrate concentrations were determined with the phenol-sulfuric acid method (Dubois et al 1956). Water solubilities were expressed as percentages on a dry matter basis.

Gel-Permeation Chromatography

A 30-mg sample of each extrudate was dispersed in 3 mL of 1*N* potassium hydroxide and stored in a refrigerator for 30 min with periodic stirring. Then 7 mL of distilled water was added. The mixture was centrifuged at 13,500 × *g* for 10 min and the supernatant was filtered through Whatman 42 filter paper. A 5-mL sample of the filtrate was chromatographed on an ascending gel-permeation chromatography (GPC) column packed with Sepharose CL-2B gel (Pharmacia-LKB, Uppsala, Sweden). A 0.02% (w/v) aqueous sodium azide solution was used as the eluant (30 mL/hr). The column was graduated in Kav units with Kav = 0 being at the point where blue dextran eluted (upper cut-off point) and Kav = 1 being at the point where potassium chloride eluted (lower cut-off point). Fractions of 2 mL were collected and assayed for carbohydrate content by the phenol-sulfuric acid method (Dubois et al 1956).

SDS-PAGE

Protein in the corn meal was separated by gel electrophoresis as described by Laemmli (1970) on a 15% polyacrylamide gel in a Mini-Protein II Dual Slab Cell gel electrophoresis apparatus (Bio-Rad, Richmond, CA). Gels were stained with Coomassie brilliant blue R-250 (0.02%, w/v), placed in methanol-water-acetic acid (40:50:10, v/v) fixative for 1 hr and destained overnight in methanol-water-acetic acid (40:50:10, v/v). For glycoprotein detection, a glycoprotein detection kit (Sigma Chemical, St. Louis, MO), which is a modification of periodic acid-Schiff (PAS) method (Zacharius et al 1969, Jay et al 1990), was used.

Statistical Design and Analysis

A 3 × 4 full-factorial treatment design was used with three barrel temperatures (100, 120, and 140°C) and four ECH contents (0, 0.5, 1, and 2%). The whole set of 12 treatments was replicated three times on three different days. Each one of the three water solubility replicate values was the mean of measurements on two sampling units. Data were analyzed using the General Linear Models procedure in SAS (Release 6.08, SAS Institute, Inc., Cary, NC) software (Littell et al 1991).

TABLE I
Water Solubility Values (%)[a] of Corn Meal Extrudates Extruded at Different Barrel Temperatures (°C) and with Different Epichlorohydrin (ECH) Contents

	Barrel Temperatures (°C)		
ECH (% db)	100	120	140
0.0	12.7 ± 4.1a–c	13.4 ± 2.3ab	14.3 ± 1.3a
0.5	11.4 ± 2.4a–d	11.7 ± 1.4a–c	11.9 ± 0.5a–c
1.0	10.2 ± 0.7b–e	9.7 ± 0.2c–e	9.7 ± 1.0c–e
2.0	7.6 ± 1.1e	9.4 ± 2.5c–e	8.0 ± 1.1de

[a] Means of three replicates ± standard deviation. Any two means followed by same letter not significantly ($P > 0.05$) different by Duncan's multiple range test.

RESULTS AND DISCUSSION

Water Solubility

Water solubility (WS) values of extrudates at various barrel temperatures and ECH contents are shown in Table I. Barrel temperature and the interaction between barrel temperature and ECH content did not significantly ($P > 0.05$) affect WS. ECH content had a significant ($P < 0.01$) negative effect on WS. Mean WS values across all levels of barrel temperature were 13.4 ± 2.5, 11.7 ± 1.4, 9.9 ± 0.7, and 8.3 ± 1.7% at 0, 0.5, 1, and 2% ECH content, respectively. The decrease in WS of ECH-containing extrudates suggested that ECH reduced starch degradation in the extruder through cross-linking between –OH groups on starch molecules.

Starch Complexing

Extrusion degrades starch molecules, affecting properties of extrudates (Davidson et al 1984, Chinnaswamy and Hanna 1990). Molecular size distribution of carbohydrates of native corn meal and corn meal extruded with and without ECH was determined with GPC (Table II). At 100°C barrel temperature, the peak for extruded corn meal without ECH shifted slightly, and carbohydrate contents in the range of Kav = 0.1–0.75 increased when compared with native corn meal. However, for corn meal extruded with ECH, in spite of the decrease in those peaks with increasing ECH content, carbohydrate content between Kav = 0.1 and 0.75 did not increase. This suggested that the decrease in high molecular weight fractions was not due to starch degradation but rather due to insolubilization of reaction products. At 140°C barrel temperature, although similar GPC patterns were obtained, carbohydrate content in the range of Kav = 0.1–0.75 was lower than that at 100°C barrel temperature (Table II). Probably, this was due to occurrence of more starch-protein complexing at the higher barrel temperature of 140°C. At both 100 and 140°C barrel temperatures, carbohydrate contents at Kav > 0.75 did not change appreciably as a result of ECH addition. This suggested that ECH reacted more with high molecular weight carbohydrates than with low molecular weight carbohydrates. Jane et al (1992) also reported that amylopectin was more susceptible to cross-linking than amylose in corn starch treated with ECH at 25°C.

Protein Complexing

As mentioned, zein is relatively hydrophobic. However, zein contains small amounts of polar amino acids with –OH functional groups (i.e., serine, threonine, and tyrosine at levels of 4.5, 2.6,

TABLE II
Carbohydrate Contents of Gel Permeation Chromatography (GPC)[a] Fractions (mg) of Corn Meal Extrudates Extruded at Different Barrel Temperatures (°C) and with Different Epichlorohydrin (ECH) Contents

	Kav < 0.1		Kav 0.1–0.75		Kav > 0.75	
ECH (% db)	100°C	140°C	100°C	140°C	100°C	140°C
0.0	1.11	0.90	1.47	0.90	0.44	0.42
0.5	0.74	0.72	1.43	0.89	0.41	0.37
1.0	0.52	0.40	0.97	0.72	0.35	0.34
2.0	0.21	0.25	0.74	0.34	0.31	0.47
Native corn meal	1.25		0.54		0.38	

[a] GPC column was graduated in Kav units with Kav = 0 being at the point where blue dextran eluted (upper cut-off point) and Kav = 1 being at the point where potassium chloride eluted (lower cut-off point).

and 4.3 g/100 g of protein, respectively) (Paulis 1981). It may be possible for these –OH groups to become cross-linking sites for formation of ECH-induced zein-zein and zein-starch complexes. SDS-PAGE was performed to detect any changes in corn protein. Figure 1A shows the results at 100°C barrel temperature. Similar results were obtained at 140°C barrel temperature (*data not shown*). Compared with native corn meal, a few protein bands (>66 kDa) of corn meal extruded without ECH disappeared. However, because increases in protein bands in the low molecular range were not observed, it was considered that protein fragmentation did not occur during extrusion. ECH did not seem to affect the main protein band of 22 kDa, which corresponds to α-zein (Wallace et al 1990). However, two protein bands of ≈29 and 17.5 kDa obviously decreased with increasing ECH content and a new protein band appeared at ≈45 kDa (indicated by arrows in Fig. 1A) in samples containing 1 or 2% ECH. This suggested that ECH was involved in some protein cross-linking in addition to starch cross-linking. The decreasing protein bands of 17.5 and 29 kDa most likely corresponded to β-zein and γ-zein, respectively, because molecular weights of 17–18 kDa for β-zein and 27 kDa for γ-zein have been reported (Esen 1987).

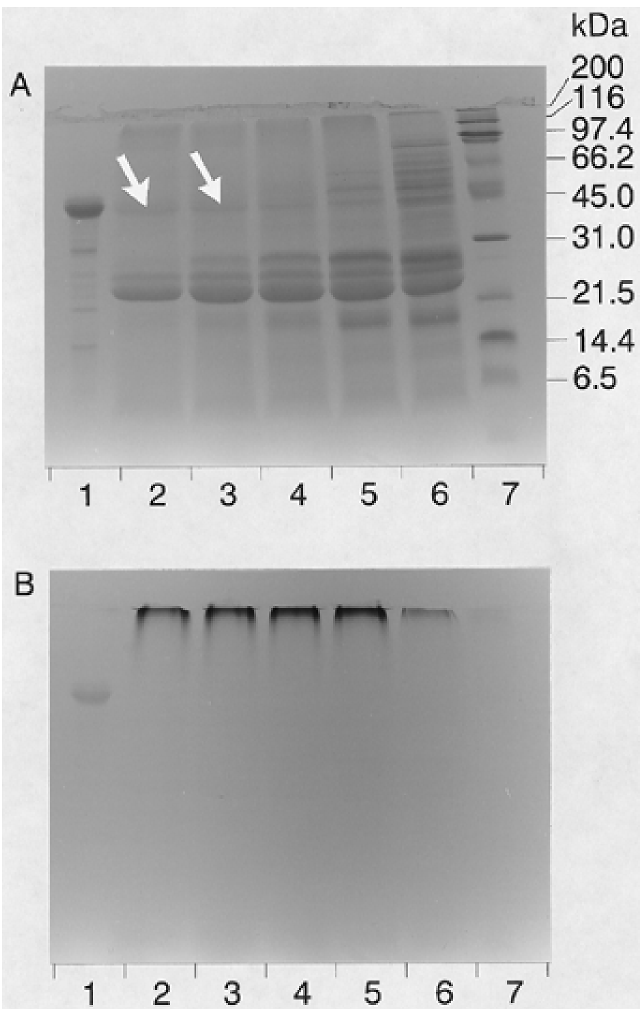

Fig. 1. SDS-PAGE patterns for native corn meal and corn meal extruded with various contents of epichlorohydrin (ECH) contents at 100°C barrel temperature. Gels were stained with Coomassie brilliant blue for corn meal protein detection **(A)** and periodic acid-Schiff (PAS) for glycoprotein detection **(B)**. Lane 1: horseradish peroxidase as a positive control for PAS staining; lanes 2–5: corn meal extruded with 2, 1, 0.5, and 0% ECH, respectively; lane 6: native corn meal; and lane 7: molecular weight standard.

Starch-Protein Complexing

To detect existence of starch-protein complexes, glycoprotein staining in SDS-PAGE was performed (Fig. 1B). The region ≈100 kDa was stained with PAS reagents and the existence of carbohydrate was revealed. However, a band corresponding to the glycoprotein molecular weight standard that was evident in Fig. 1A did not appear in Fig. 1B. Therefore, existence of a glycoprotein or a starch-protein complex could not be concluded.

Implication

This study indicated that addition of 1–2% ECH before extrusion processing can reduce water solubility of corn meal extrudates, thereby possibly improving their functionality as loose-fill packaging materials. Occurrence of ECH-induced cross-linking involving both starch, mainly amylopectin, and protein was suggested by GPC and SDS-PAGE results. More analytical testing is needed to further study and conclusively identify covalent crosslinks within ECH-containing starch-based extrudates.

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