Relationship Between Popcorn Composition and Expansion Volume and Discrimination of Corn Types by Using Zein Properties

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ABSTRACT

Cereal Chem. 83(1):86–92

The objective of this study was to determine the relationship between the amount and type of lipids, starch composition and structure, and storage proteins on popcorn expansion and to evaluate whether popcorns could be discriminated from other types of corn based on the protein elution parameters. Seven commercial Argentinean popcorn samples were used in the study and significant differences were observed in the popping volume of these popcorns. A significant negative correlation was observed between oleic acid and popping volume and a positive correlation was observed between linoleic acid and popping volume. Popcorn starch properties were significantly different from normal corn but no particular measured attribute of starch correlated with popping volume. α-Zein proteins and glutelins significantly correlated with popcorn expansion volume with R^2 = 0.963 and 0.744, respectively. The elution patterns of corn proteins could also be used to discriminate between different types of corn including popcorn, dent, and flint corns.

Expansion volume is the most critical quality factor for popcorns. Numerous studies have investigated the influence of a variety of parameters on popping volume and popping time. Several studies have reported on the effects of genotype (Song et al 1991; Kandala et al 1994; Tian et al 2001), kernel density (Park and Maga 2002), kernel size (Song et al 1991), kernel damage (Park and Maga 2002; Singh et al 2004), moisture content (Song and Eckhoff 1994a,b), and storage condition (Park and Maga 2002) on the expansion volume of popcorns. Park et al (2000) reported on the chemical composition of several popcorn hybrids. They reported that popcorn had lower crude fat content and sugar content, higher amylose-to-amylopectin ratio, and higher levels of linoleic and linolenic acids than normal corn starch. However, none of the studies have investigated the potential relationship between popcorn compositions on popping volume.

Endosperm expansion is highly related to the texture of the endosperm matrix. Horny endosperm corns result in popping expansion values greater than floury endosperm corn (Hoseney et al 1983; Rooney and Serna-Saldívar 1987). Endosperm texture, in turn, was associated with the amount and type of zein proteins (Dombrink-Kurtzmann and Bietz 1993; Eyhérabide et al 1996). The endosperm matrix proteins also contain other classes of proteins including the glutelins (Wilson 1987) that might influence endosperm texture of corn. Moreover starch composition might play a role in the expansion properties of popcorn. It is reported that starch amylose and amylopectin have a greater impact on extrusion expansion of waxy corn with superior expansion properties in the extrusion process than either normal or high-amylose corn (Mercier and Feillet 1975; Bhattacharya and Hanna 1987; Zhang and Hoseney 1998). Thus it is likely that starch composition influences expansion properties in the popping process. No reference was found regarding fatty acid composition of the oil and its relationship with popping expansion of the corn. However, studies in our laboratory (*unpublished data*) suggest a certain degree of interaction between unsaturated lipids and starch as a consequence of the extrusion process.

Characterization of the storage proteins of corn by reversed-phase HPLC can also be used to classify corns by type. Classification by type is based primarily on phenotypic traits, which may be affected by environment and thus may not accurately express genetic characteristics. An accurate description of corn samples for both research and marketing reasons is sometimes necessary (Almeida et al 1997; Hardeep et al 2001; Hojilla and Johnson 2003; Singh et al 2004). Therefore we attempted a classification of different corn samples by means of zein chromatograms trying to discriminate between popcorn types and other types of corns. Robutti et al (2000) could classify several corn samples into racial types by multivariate analysis of zein RP-HPLC data.

The purpose of the present study, therefore, was to determine the relationships between the amount and type of lipids, starch composition and structure, and storage proteins (zeins and glutelins) on the expansion capacity of popcorn and to discriminate between popcorns and other types of corn by zein proteins.

MATERIALS AND METHODS

Materials and Popping Expansion Procedure

Seven commercial Argentinean popcorn samples were used in this study. The popcorn samples were from open-pollinated cultivars and selected based on low, medium, or high expansion values. Kernels were harvested and tempered to 14% moisture content on a dry basis.

A metric weight volume tester (MWVT) was used as a volumetric expander (Cretors, Valley Popcorn Co., Neenah, WI) operated at 1,400W to pop the samples. The popped volume of 250 g of corn sample was measured. The equipment was warmed by popping kernels two to three times before test samples were popped. Sample mixed with 100 mL of coconut oil was added to the pan when the pan temperature reached 480°F and was covered. Corn samples were popped until the time between popping was 5 sec. The popped corn was quickly transferred to a measuring cylinder and the expansion at the highest point, without shaking was then recorded. MWVT is the measurement of cubic centimeters of popped corn/g of unpopped corn and was measured by using the approved procedure described in the equipment operational manual. The analyses were conducted in duplicates and the average values reported.

Lipid Analysis

Ten kernels/sample were randomly selected and homogenized. The oil from ≈200 mg of flour was extracted in hexane. The

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DOI: 10.1094/CC-83-0086
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alternate method for the preparation of fatty acid methyl esters was performed as described in Official Method Ce 2-66 (AOCS 1997). A gas chromatograph (Aerograph model 2740-10, Varian Instrument Division, Palo Alto, CA) equipped with a flame-ionization detector and a stainless steel Supelco GP 10% SP-2330 column (cyanosilicone phase) in Chromosorb WAW 100/120 mesh 1/8 i.d. (Supelco, Bellefonte, PA) was used. The column temperature was set at 200°C, and the injector and detector ports were set at 220°C. The flow rate for the N_2 carrier gas was 30 mL/min and the detector gases were set at 30 mL/min for H_2 and 300 mL/min for air. Peak areas were measured by using a Hewlett-Packard model 3365A integrator. The relative amounts of 16:0, 18:0, 18:1, and 18:2 in total lipids were determined as a percentage of the total peak area. The data were mean values of duplicate determinations

Starch Analysis

Dispersed starch was prepared according to the procedure of Klucinec and Thompson (1998) with modification. Ground popcorn (4 g) was soaked in 30 mL of 0.45% sodium metabisulfite and stirred at 50°C in a water bath for 24 hr. The slurry was homogenized and then centrifuged. The precipitate was washed four times with 30 mL of 70% ethanol. The sample was then boiled in 40 mL of 90% DMSO for 3 hr with constant stirring, centrifuged, and precipitated using ethanol. The precipitated starch sample was then washed with acetone and dried overnight at room temperature. The dried dispersed starch sample was used for further analyses.

The amylose and amylopectin distribution profile were determined by using a Sepharose CL-2B SEC column (73 cm × 2.5 cm; Pharmacia Fine Chemicals, Uppsala, Sweden) flowing against gravity (Klucinec and Thompson 1998). A 0.04% (w/v) sodium hydroxide solution (pH 12) containing 0.02% (w/v) sodium azide was used as the mobile phase. Dispersed starch samples (15 mg) were further dispersed using 0.4% sodium hydroxide overnight and diluted with 5 mL of deionized water before being loaded onto the column. Eluate (500 mL) was collected with 5 mL as one fraction for each popcorn sample. Total carbohydrate content in each 500-mL eluate fraction was determined using the phenol-sulfuric acid assay procedure of Dubois et al (1956). The analysis was not replicated becaused the data did not correlate with popping volume.

Starch samples were debranched by using the procedure described by Hizukuri et al (1981). Samples were dispersed in 90% DMSO and debranched using isoamylase (Megazyme, Ireland; 0.5 µg/mL in 0.05*N* NaAc buffer, pH 3.75). The debranched samples were analyzed using the procedure described by Klucinec and Thompson (1998) on a system consisting of a pump (model 515 solvent delivery system; Waters Corp., Milford, MA) and a refractive index detector (model 2414; Waters). Two 30-cm columns packed with 6-µm porous silica microspheres connected in series were used for the separations. The mobile phase in the system was 100% DMSO with flow rate 0.5 mL/min. The analysis was not replicated because the data did not correlate with popping volume.

The blue value of starch samples were determined using the procedure described by Morrison and Laignelet (1983). Dispersed starch (40 mg) was redispersed in a mixture of 9 mL of 90% DMSO and 1.0 mL of 6*M* urea and boiled for 3 hr with intermittent stirring. A 1.0-mL aliquot of each sample was added to a 100-mL volumetric flask and mixed with 2.0 mL of an aqueous I_2-KI (2 mg of I_2/mL and 20 mg of KI/mL) solution and filled to the mark. Blank solutions were made similarly without adding starch. All solutions were stored in darkness for 15 min before the measurement. The blue value was defined as absorbance at 635 nm. The data are mean values of duplicate observations.

Thermal properties of samples were evaluated by using a differential scanning calorimetry (Pyris 1, Perkin-Elmer). Ground sample (1:2, sample-to-water) was scanned from 20 to 140°C at 10°C/min as described by Seetharaman et al (2004). All analyses were conducted in duplicates and average values reported.

Protein Analysis

Kernels (30–40) of each sample were milled for 30 sec in an analytical mill with a refrigerated stainless steel chamber (HQ analyzer model MC-II, Control Química, Buenos Aires, Argentina) to a final particle size of ≈250 µm. All organic solvents were HPLC-grade and other chemicals were reagent grade. For the classification experiments, zein chromatograms taken from a previous work (Robutti et al 2000) were added to the chromatograms of the seven popcorns described above. Chromatograms used in the analysis included two different samples each of Cristalino Colorado (red flint) type, Dentado (dent) type, and Pisingallo (popcorn) type. Classification was run on a total of 13 samples.

All extractions were conducted in 10-mL capped polypropylene centrifuge tubes. Milled sample (200 mg) was extracted twice (2 and 1 mL) for reduced zeins from duplicate samples with 70% ethanol and 0.5% sodium acetate (Na Ac) + 0.2% dithiothreitol (DTT) for 1 hr using a vortex mixer at room temperature. Extracts were then clarified by centrifugation at 3,000 × *g* for 30 min. Combined supernatants were transferred to vials for HPLC analysis (HP model 1050) after filtration (0.22 µm). Reduced and nonalkylated glutelins subunit samples were prepared by reextracting the residues remaining after zein extraction with 3 mL of 5*M* urea + 0.05*N* sodium phosphate (pH 7.7) + 0.5% DTT under nitrogen at 30°C for 2 hr with a vortex mixer as above and centrifuged at 16,000 × *g* for 30 min at 8°C. Insoluble materials can also be removed by filtration (0.22 or 0.45 µm) and placed into autosampler vials. This glutelin extraction procedure was suggested by F. Huebner (*personal communication).*

A standard column (4.6 × 250 mm) with a pore size of 300Å (Vydac C_{18}, Separations Group, Hesperia, CA) was preceded by a precolumn filter with a 2-µm frit (A-430 2U filter, Upchurch, Oak Harbor, WA). To enhance resolution, the column was maintained at 60°C. Solvents for both protein separations were acetonitrile (ACN) (Solvent B) and distilled water (Solvent A), which was further purified (NANOpure, Barnstead) and autoclaved both solvents contained 0.1% trifluoroacetic acid (TFA). Chromatographic data was imported into GRAMS32 (Galactic Industries, Salem, NH) format. Chromatograms were truncated to leave retention times from 20 to 50 min for zeins and 5 to 25 min for glutelins. Multivariate analysis was performed using the PLS/IQ add-on application.

Statistical Analysis

Chromatographic data was imported into GRAMS32 and analyzed by principal component analysis (PCA), a multivariate approach designed for multicorrelated data using the PLS/IQ add-on. This method projects the information contained in the original intercorrelated variables onto a smaller set of new variables called principal components (PC). PC are linear combinations of the original variables, which in our case are the absorbance readings. Each chromatogram consists of ≈1,800 readings. The first PC explains the highest percentage of the variability within the samples under study, the second PC explains the next highest percentage and so on. Usually the first two or three PC retrieve a high enough percentage of the information contained in the whole set of original variables. The equation relating PC and variables is PC = L1A1 + L2A2 +…….+ LnAn, where the L is a loading and A is an absorbance value at different retention times. The larger the L value, the larger the influence of the protein eluting at this retention time on the score value of the PC. Then a model is developed to predict a parameter (popping volume) from the PC. The equation relating the variables is PV = K1PC1 + K2PC2 +….+.K*n*PC*n*., where PV is the popping volume, K is a regression coefficient and PC1–*n* are the principal component scores. The predicted values are then regressed to the corresponding measured values to check the validity of the correlation.

RESULTS AND DISCUSSION

Expansion Values

Significant differences were observed in the expansion values of the different commercial popcorn cultivars and expansion values were 35.5 cm^3/g to 46.75 cm^3/g.

Lipids

The fatty acid content of the commercial popcorn cultivars are shown in Table I. The lipid content of popcorns primarily consisted of linoleic acid (56.9–63.4%), followed by oleic acid (23.3–29.7%), palmitic acid (9.1–12.1%), and stearic acid (1.2–1.9%). These values are similar to those reported by Park et al (2000) for popcorn hybrids from the United States, although the range of values observed for each fatty acid in the Argentinean cultivars was slightly broader than the U.S. cultivars tested by Park et al (2000).

A significant correlation was observed between expansion values and oleic ($R = -0.702$; $P < 0.005$) and linoleic ($R = 0.612$; $P < 0.02$) acids (Table I). The correlation coefficients for the saturated acids were much lower. This would suggest an interaction of the more unsaturated lipids with other endosperm components, more specifically an interaction with amylose, thereby affecting the expansion properties of popcorns. Karkalas et al (1995) reported that ≈60% of the amylose lipid complex in native starch granules is with linoleic acid. It is likely that the amylose-lipid complex, which decreases granule swelling and increases gelatinization temperature (Morrison 1995), possibly plays a role in increased popping volume of popcorn. Further studies are required to clarify this hypothesis.

Starch Properties

Starch molecular distribution is shown in Table II. Although these analyses were not replicated, prior experience with this procedure in our laboratory has shown consistent results with very low standard deviation. Therefore, these results and results for debranched starches are qualitative in their description of the polymer attributes of popcorn starch. To the best of our knowledge, such data have not been reported for popcorn starches.

All starches displayed two peaks, typically classified as amylopectin (AP) and amylose (AM), with a higher proportion of amylopectin fraction than amylose for ordinary corn (Han et al 2003). However, differences were observed for the amylose and amylopectin contents for different starch samples. The amylose and amylopectin contents of the starches were determined by using GPC based on K' values; $K' < 0.193$ indicates AP fraction and $K' > 0.193$ indicates AM fraction. Starch from popcorn sample 5 had the highest AP and lowest AM content, while starch from popcorn sample 1 had the lowest AP and highest AM levels among the different popcorn samples. The AP contents of all the popcorn cultivars were lower than those observed for ordinary corn, and the corresponding AM contents were higher for all popcorn cultivars compared with ordinary corn. The AP and AM contents measured using GPC showed no correlation with expansion volume of popcorns.

The HP-SEC chromatograms of the debranched starch samples were divided into three regions based on the retention times of two response minima for debranched starch from normal corn. Chromatography of selected popcorn samples and normal corn are shown in Fig. 1. Hizukuri (1986) reported HP-SEC chromatograms using improved columns and techniques showed tetramodal distribution profiles for potato, tapioca, and kuzu amylopectin. Yuan et al (1993) used a high-pressure column (Zorbax PSM) and obtained a trimodal distribution from waxy corn starch. Our study indicated that the amylopectin of popcorn did not show a trimodal distribution and only exhibited a bimodal distribution similar to that of normal corn.

Amylose content of commercial popcorn samples was 30.5–38.0%. The amylose contents of popcorn samples were higher than ordinary corn used in this study. The area distribution and DP_n calculations are shown in Table III. For common corn starch, regions I, II, and III correspond to 22.5, 22.8, and 54.7% total area, respectively. Starches from the different commercial popcorn samples had significantly higher area for region I and lower area for regions II and III compared with ordinary corn used in this study. The DP_n for region I of the popcorn starches was 230–248, while the DP_n values for regions II and III were 41 and 13–14, respectively. All the DP_n values were significantly lower than that

TABLE I
Fatty Acid Composition and Expansion Data for Kernels from Seven Commercial Popcorn Cultivars

Popcorn Sample	Palmitic Acid (16:0)	Stearic Acid (18:0)	Oleic Acid (18:1)	Linoleic Acid (18:2)	Expansion (cm^3/g)
1	11.1c[a]	1.9a	25.9c	61.1b	45.5ab
2	10.9d	1.4cd	27.6b	60.3c	38.5cd
3	10.6d	1.8a	27.5b	59.8c	35.5d
4	11.6b	1.2d	29.5a	57.8d	41.8bc
5	9.1e	1.6b	25.9c	63.4a	41.8bc
6	12.1a	1.3d	29.6a	57.1e	35.8d
7	11.9a	1.5bc	23.2d	63.4a	46.8a

[a] Values followed by different letters in columns indicate significantly different means at $P < 0.05$.

TABLE II
Amylose and Amylopectin Content of Popcorn Starches Measured by Two Different Procedures and Blue Value of Popcorn Starches

	GPC[a]		HP-SEC[b]		
Sample	AM (%)	AP (%)	AM (%)	AP (%)	Blue Value
1	44.9	55.1	32.2	67.8	0.392f[c]
2	42.3	57.7	38.0	62.0	0.511a
3	39.7	60.3	31.2	68.8	0.402e
4	39.8	60.2	33.8	66.2	0.417c
5	38.4	61.6	30.5	69.5	0.407d
6	40.0	60.0	34.7	65.3	0.441b
7	41.4	58.6	31.8	68.2	0.414c
Ordinary corn	33.9	66.1	22.5	77.5	0.410c

[a] Gel-permeation chromatography.
[b] High-pressure size-exclusion chromatography.
[c] Values followed by different letters in columns indicate significantly different means at $P < 0.05$.

observed for common corn starch for all three fractions. The result of amylose and amylopectin showed a similar trend compared with ordinary corn when measured by GPC and HP-SEC with different values. Klucinec and Thompson (1998) reported that high-amylose corn starches contained higher levels of intermediate-type starch polymers that had longer external chain lengths in AP than common corn starch. It is likely that popcorn, which is more similar to high-amylose corn than common corn, has properties similar to high-amylose corn, thus influencing the accurate determination of amylose content using different procedures.

Blue value of starches from the commercial popcorn cultivars were significantly different at 0.392–0.511 (Table IV). Although a poor correlation was observed between blue value and expansion ratio, the removal of popcorn samples 2 and 3 resulted in a value of $R = -0.728$ ($P < 0.017$). Blue values are positively associated with amylose content (Suzuki et al 1992). Although the research of Chinnaswamy and Hanna (1988) indicated that high-amylose content corn starch has a higher expansion ratio during extrusion. Other researchers (Mercier and Feillet 1975; Bhattacharya and Hanna 1987) suggested that waxy corn showed superior expansion properties. Our study indicated that blue value, amylose, and amylopectin content did not significantly correlate with popcorn expansion.

The DSC thermogram of the common corn starch showed two typical endothermic peaks, with a starch gelatinization peak at ≈70°C and the melting peak of the amylose-lipid complex at ≈100°C. The DSC thermogram of the commercial popcorn cultivars exhibited only one peak at ≈75°C with a broad and significant shoulder with a peak at ≈100°C (Fig. 2). The thermal properties of popcorn are shown in Table V. The onset temperature of gelatinization and the enthalpy of gelatinization of the popcorn samples were similar to that observed for normal corn. However, the onset temperature of the amylose-lipid peak melting event during the rescan was significantly higher for popcorn samples compared with the normal corn sample. Furthermore, the enthalpy of melting of the amylose-lipid peak during the rescan was significantly lower in the popcorn samples compared with the normal corn sample.

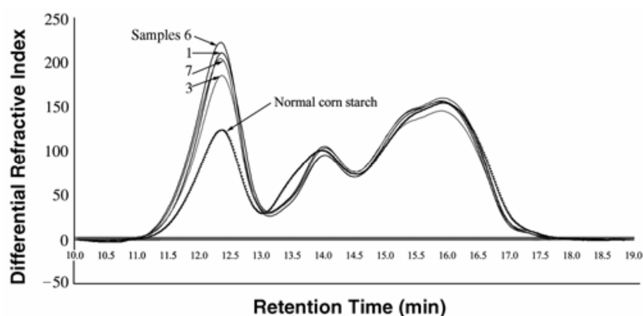

Fig. 1. High-performance size-exclusion chromatography of isoamylase-debranched nongranular normal corn and selected popcorn starches based on highest and lowest expansion ratio.

Because the results from this study suggested no relationships between starch molecular properties and expansion ratio, the other compositional attributes of popcorn are possibly responsible for the expansion ratio properties of popcorn. The research of Wolf et al (1975) indicated that protein and amino acid composition compositions were different for corn with different amylose content. Reeve and Walker (1969) also reported that differences in distribution of horny and floury endosperm and differences in protein content influence the capacity to expand. Other than starch and protein compositions, the kernel size and genotype also significantly affected the popping volume (Song et al 1991; Zhang and Hoseney 1998).

Protein Properties

The chromatograms of zein and glutelin proteins are shown in Figs. 3 and 4, respectively. A model was developed to predict popping values from zein and glutelin chromatograms. The best R^2 value for the regression between predicted and actual popping values was obtained using one factor (the first PC). Thus the equation was $PV_P = K_1PC_1$, where PV_P is the predicted popping volume. A value of $R^2 = 0.963$ was found for the regression between actual values and those predicted from the zein chromatograms. The main loadings for the first factor of the prediction model were peaks at ≈41.8 and 42.9 min (Fig. 5), thus indicating that those zeins were most associated with the expansion properties of the corn. Those retention times correspond to the region of α-zeins or zein-1 (Z1) which has been associated with endosperm hardness (Eyherabide et al 1996). This endosperm property is thought to be responsible, at least in part, for the expansion values of popcorn (Hoseney et al 1983; Rooney and Serna-Saldívar 1987). To corroborate these findings, a correlation was made between the absorbance of the different samples at 42.9 min and the actual popping volumes. A value of $R = -0.707$ indicated that the zein eluting at this particular time had a negative association with popping volume. The zeins eluting between ≈22 and 28 min (zein-2 or Z2) were also associated with endosperm hardness (Dombrink-Kurtzmann and Bietz 1993; Eyhérabide et al 1996). In this study, however, we found very little association between Z2 and expansion properties. For the regression between actual popping values and values predicted from the model based on glutelins, the $R^2 = 0.744$; somewhat lower than the one for zeins. The main loadings for the first factor in this case were found at ≈13.7 and 11.7 min. Again a correlation was made between the absorbance of the different samples at 13.7 min and the popping volumes. In this case, the $R = 0.567$, thus indicating a certain degree of a positive association of this particular glutelin with

TABLE IV
Correlation Coefficients Between Popcorn Popping Volume, Fatty Acid Content, and Blue Value

	Blue Value	Blue Value[a]	Oleic Acid	Linoleic Acid
Correlation coefficient	–0.340	–0.728	–0.702	0.612
P value	0.234	0.017	0.005	0.020

[a] Correlation coefficient value excluding data for popcorn samples 2 and 3.

TABLE III
Debranched Starch Properties

Popcorn Sample	Peak 1 (%)	Peak 2 (%)	Peak 3 (%)	Avg DP_n Peak 1	Avg DP_n Peak 2	Avg DP_n Peak 3
1	32.2	19.2	48.6	230	41	14
2	38.0	17.2	44.7	248	41	13
3	31.2	19.3	49.0	236	41	13
4	33.8	18.1	47.4	241	41	14
5	30.5	18.5	50.0	230	40	13
6	34.7	17.4	46.8	243	41	14
7	31.8	18.8	48.0	241	41	14
Ordinary corn	22.5	22.8	54.7	251	50	15

TABLE V
Thermal Properties of Popcorn and Normal Corn Samples

Popcorn Sample	T_o (°C)[a]	ΔH (J/g)[b]	Rescan T_o (°C)[c]	Rescan ΔH (J/g)[d]
1	66.4 ± 0.26	8.9 ± 0.03	91.5 ± 0.14	0.29 ± 0.02
2	67.6 ± 0.33	8.9 ± 0.07	91.5 ± 0.17	0.29 ± 0.06
3	65.9 ± 0.16	9.2 ± 0.23	91.4 ± 0.25	0.33 ± 0.03
4	67.0 ± 0.30	8.9 ± 0.10	91.4 ± 0.10	0.32 ± 0.01
5	64.5 ± 0.06	8.4 ± 0.15	91.2 ± 0.25	0.23 ± 0.02
6	67.8 ± 0.38	10.3 ± 0.59	91.5 ± 0.13	0.30 ± 0.02
7	65.0 ± 0.77	9.0 ± 0.27	91.4 ± 0.11	0.33 ± 0.01
Ordinary corn starch	66.9 ± 0.27	9.1 ± 0.35	79.9 ± 0.63	0.81 ± 0.17

[a] Onset temperature of gelatinization.
[b] Enthalpy of gelatinization.
[c] Onset temperature of amylose-lipid complex during rescan.
[d] Enthalpy of melting of amylose-lipid complex during rescan.

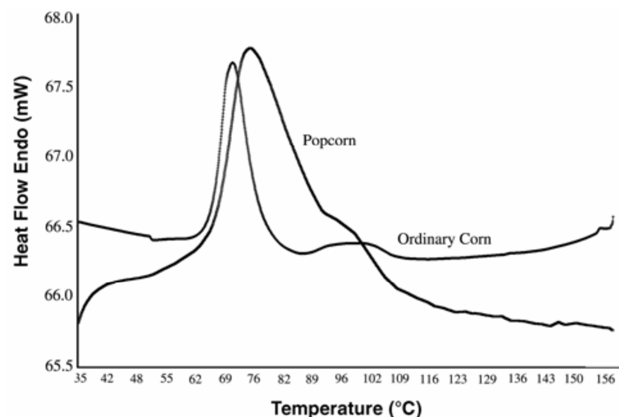

Fig. 2. Differential scanning calorimetry endotherm curve of popcorn and ordinary corn.

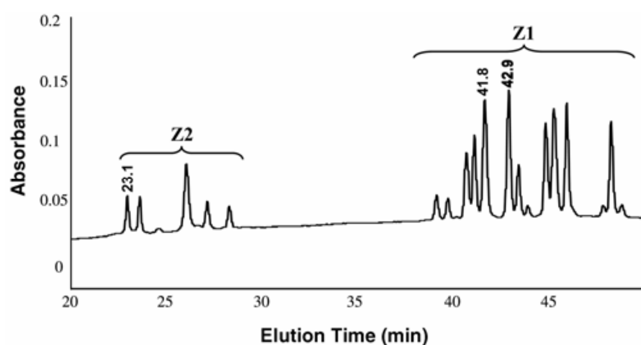

Fig. 3. Reversed-phase high-performance liquid chromatography analyses of reduced zein popcorn samples. Gradient 28% (solvent B) initially, increasing linearly to 39.5% over 50 min, held at 39.5% for 10 min, and then reequilibrated at 28% for 10 min. Flow rate 1.0 mL/min. Sample volume 5 µL.

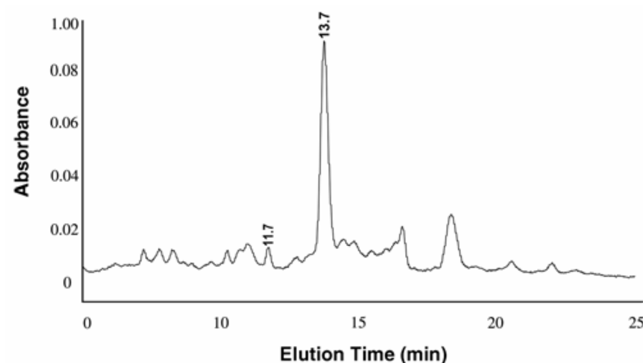

Fig. 4. Reversed-phase high-performance liquid chromatography analyses of reduced nonalkylated glutelin popcorn samples. Gradient 30% (solvent B) initially, increasing linearly to 35% at 6 min, then to 40% at 11 min, and finally to 46% at 25 min. Reequilibration during 10 min at 30%. Flow rate 1.0 mL/min. Sample volume 25 µL.

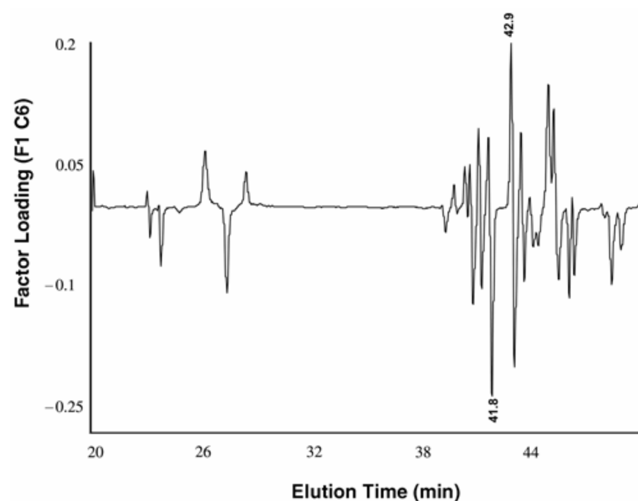

Fig. 5. Loading for first factor of the model for predicting expansion from zein chromatogram.

popping volume. Horny endosperm corn result in greater popping expansion values than floury endosperm corn (Hoseney et al 1983; Rooney and Serna-Saldívar 1987) and endosperm texture, in turn, has been associated with the amount and type of zein proteins (Dombrink-Kurtzmann and Bietz 1993; Eyhérabide et al 1996). Therefore the association between specific proteins and expansion values are thought to be a result of the influence of these proteins on endosperm hardness. With respect to glutelins, to the best of our knowledge, no report exists on the association between these proteins and endosperm hardness. However recent work at our laboratory (*unpublished data*) suggests a certain degree of positive association between glutelins eluting at 14.7 min by RP-HPLC and endosperm hardness in corn.

There is specific kernel storage proteins (zeins and glutelins) associated (positively or negatively) with the expansion values of popcorn, possibly in a functional manner. If this was so, selection for improved expansion values could be performed by increasing (or decreasing) the proportion of those proteins according to the sign of the association (+*R* or –*R*).

Corn Type Classification

For the classification experiments, the chromatograms taken from Robutti et al (2000) were added to the seven chromatograms

TABLE VI
Mean Comparison of Absorbance at 23.1 min Among Three Different Types of Corn

Sample	Type	Absorbance @ 23.1 min	Sample	Type	Absorbance @ 23.1 min	Sample	Type	Absorbance @ 23.1 min
1	Popcorn	0.0585	9	Flint	0.061	14	Dent	0.1155
2	Popcorn	0.055	11	Flint	0.0685	15	Dent	0.2356
3	Popcorn	0.0725						
4	Popcorn	0.0775						
5	Popcorn	0.051						
7	Popcorn	0.043						
12	Popcorn	0.04						
13	Popcorn	0.0475						
Mean	Popcorn	0.0556	Mean	Flint	0.065	Mean	Dent	0.340

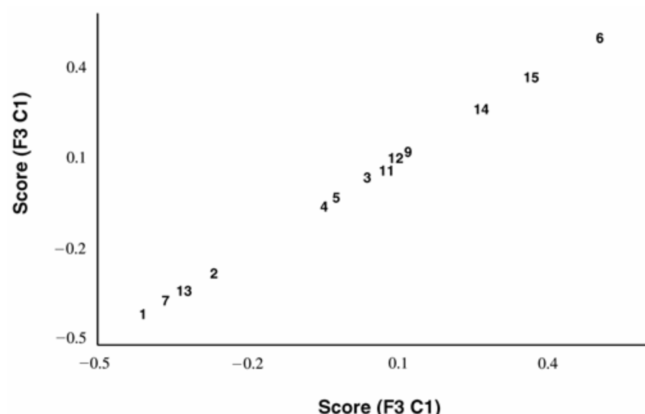

Fig. 6. Grouping of corn types based on principal component analysis of zein RP-HPLC data.

from this work and a multifile was created and PCA software was run. Factor 3 differentiated quite well among types (Fig. 6). Samples 1–7 are from this study, samples 9–11 are flint corns, samples 12 and 13 are additional popcorn cultivars, and samples 14 and 15 are dent corns. Overlapping is observed with sample 12 (popcorn) located between samples 9 and 11, which are both flint. Sample 6 is located very far from all others. The main loadings for factor 3 were at 23.1 and 42.9 min. Based on the findings reported above, we speculated that the zein eluting at 42.9 min discriminated the popcorns on the basis of popping volume, whereas the one eluting at 23.1 discriminates on the basis of the corn type (flint, dent, and pop). To corroborate the second assumption, we ran a Student *t*-test for the means of the absorbance at 23.1 min of each type of corn (Table VI). Though no statistically significant differences were found among mean values, a trend can be observed for the mean values increasing from popcorn to flint to dent, which is essentially the order in which samples are arranged in Fig. 6.

The absorbance for sample 6 was not included in the mean value of popcorns because of its unusually large value (0.105). We speculate this large value is the cause of sample 6 being so far removed from all others in Fig. 6.

CONCLUSIONS

Significant differences were observed in the popping volume of the seven commercial Argentinean popcorn samples used in this study. A significant negative correlation was observed between oleic acid and popping volume and a positive correlation was observed between linoleic acid and popping volume. Popcorn starch properties were significantly different from normal corn but no particular measured attribute of starch correlated with popping volume. α-Zein proteins and glutelins significantly correlated with popcorn expansion volume with R^2 = 0.963 and 0.744, respectively. The elution patterns of corn proteins could also be used to discriminate between different types of corn, including popcorn, dent and flint corns.

ACKNOWLEDGMENTS

We gratefully acknowledge the assistance of Ana Di Martino in the preparation and edition of the chromatogram figures.

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[Received April 29, 2005. Accepted August 4, 2005.]