2003 MAFMA Final Report

Project Title: **Suitability of Maize for Dry Milling and Extrusion Processing**

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Objective Summary

We developed a decision matrix for selecting maize hybrids that are best suited for dry milling and extrusion processing using physical, biochemical, and structural factors.

Objective Accomplishments

a. Classification and prediction of maize quality and hardness-associate properties

Maize hybrids grown at different locations in Kansas and Missouri and harvested manually during 2002 and 2003 were used for this project. Several quality and hardness associated properties were measured using 248 maize hybrids with moisture content 13±1%. The hybrids were scanned using near-infrared transmittance (NIT) to collect whole spectra and measure the specific absorbance at 860 nm as an indicator of maize hardness. Multivariate statistical analyses, such as principal component analysis (PCA), factor analysis (FA), cluster analysis (CA), and discriminant analysis (DA) were employed to create the classification of maize hybrids into several subgroups representing unique hardness-associated physical and chemical properties and to assign the unknown samples into predetermined groups by utilizing physical, chemical, and spectral data.

PCA results revealed that two principal components could explain the total variability of data. First principal component was composed of physical and spectral hardness-associated properties, including test weight (TW), NIT density, Tangential Abrasive Dehulling Device test (TADD), air comparison pycnometer density (DEN_P), and the absorbance at 860 nm (W860), whereas second principal component showed higher association among chemical constituents starch, protein, and oil contents. Since fewer principal components contain almost all information in original variables, principal component scores could be used for cluster analysis and discriminant analysis. The normality and outliers of samples were checked by PROC UNIVARIATE procedure using SAS (Release v 8.1, Cary, NC). Four outliers that significantly affected the number and distribution of clusters in cluster analysis were determined and subsequently eliminated by looking at the stem-and leaf plot of UNIVARIATE result as well as graphical plot created by first principal component scores.

Two factor analyses, principal FA and maximum likelihood FA, were performed after PCA. Two largest eigenvalues were observed in principal factor analysis. Thus, two factors were initially used for maximum likelihood factor analysis to determine the final adequate number of factors. Two factors were considered to be rather insufficient to account for total variability of data because Akaike's Information Criterion (AIC) and Schwarz's Bayesian Criterion (SBC) in which smaller values indicate a better choice of the number of factors exhibited higher values. Final number of factors was determined as 3 factors that showed lower AIC and SBC values and satisfactory overall root mean squares in residuals and partial correlation. Table I shows the new sets of variables of 3 rotated factors and the correlations of variables in each factor. Factor 1 seems to exhibit physical and spectral hardness-associated properties while factor 2 and 3 are likely to explain the relationship among chemical constituents of maize hybrids.

CA was able to group maize samples into several subgroups (clusters) with similar quality and hardness-associated properties characterized by PCA and FA. The median clustering algorithm performed better than other methods in grouping check samples that are the same hybrid, but planted at different locations across both years. According to a pseudo Hotelling's T^2, a pseudo F statistic, and Cubic Clustering Criterion, 7 or 10 clusters were predetermined as shown in the plot (Fig. 1). The final number of clusters was further determined by calculating Beal's pseudo F statistic (F*). Although significant difference was not found between 7 and 10 clusters in F* statistic, plot and sum of squared distance within cluster indicated that 10 clusters might classify maize samples more naturally and accurately than 7 clusters. Each cluster showed significantly different physical, spectral, and chemical properties associated with maize quality and hardness (Table II). It is expected that this approach may provide fundamental information in selecting maize hybrids for various end-use performance.

PROC DISCRIMINANT procedure in DA was performed to compute the Mahalanobis squared distance, posterior probability, and each population's (cluster's) classification function. Based on these results, the new maize samples could be classified into predetermined groups resulting from cluster analysis. The probabilities of correct classification resulting from classification function were 89% in resubstitution method for new maize samples and 87% in the cross-validation method, respectively. Misclassified samples by the discriminant function had lower means of posterior probabilities than samples classified correctly (Table III). This implies that samples would be classified with higher probabilities by creating a new discriminant function under slightly modified test conditions, for instance, increasing the number of observation in the cluster and selecting different physical and chemical tests. The prior probability option was introduced in analyzing data since each cluster has a different number of observations.

b. Physical and Biochemical Determinants of Maize Hardness and processing properties

Six maize hybrids were selected from clusters that were created by procedure as described above and represent distinct physical, spectral, and chemical quality and hardness-associated properties. These hybrids were classified into two major groups according to their overall hardness. Three hybrids (AD776W, AD792W, and T-2914) were regarded as harder maize hybrid whereas the remaining three hybrids (NK70-D5, P61-491, and A6887) softer maize hybrid. Dry milling of maize samples tempered to 18% moisture content for 45 min was performed using an Allias laboratory mill using a long flow procedure that maximizes low fat grit extraction. Dry milled grit samples for extrusion were prepared by mixing 35% fine particles and 65% coarse particles to give similar particle sizes and distributions, consequently removing the effect of particle size on the final extrudate properties. To characterize the grits and extrudates, the physical, mechanical, and biochemical properties of grits and extrudates were extensively studied.

Simple and partial correlation of selected maize hybrids

Some quality and hardness-associated properties of selected hybrids were highly and significantly correlated with the contents of endosperm protein, total zein and α- zeins but not that of γ- zeins (Table IV). Partial correlation coefficient of these properties with the contents of total zein and α- zeins were not significant at constant endosperm protein content.

RP-HPLC of grits and extrudates

Each maize hybrid had a unique RP-HPLC chromatogram as well as zein subclass composition. In chromatograms of zeins soluble in 60% t-butanol plus 2% β-mercaptoethanol, harder grits tended to have a greater amount of earlier eluting (ca. 21-25 min) zein (mainly γ-zein) and a lower content of second eluting (25-29 min) zein (mainly β-zein). The content ratio of γ-zein to (γ-zein + β-zein) was positively proportional to some hardness measurements such as TADD ($r = 0.815$, $P < 0.01$) and TW ($r = 0.810$, $P < 0.01$). However, AD776W, one of harder maize hybrid, had second lowest γ-zein content and a larger amount of α- zeins that have lower cysteine content. This implies that chemical interactions other than thiol and disulfide interactions might significantly contribute to endosperm hardness.

Protein solubility was significantly reduced after extrusion processing as observed in RP-HPLC chromatograms of reduced and non-reduced extrudates (Fig. 2). Similar results were shown in size-exclusion chromatogram. While the RP-HPLC chromatograms of the samples extracted under reducing conditions showed only minor quantitative changes, samples extracted under non-reducing conditions showed a much greater degree of change after extrusion. The chromatograms of non-reduced samples (mainly α-zeins) were divided into two areas, area 1 (Zα1) eluting between 37 and 41 min, and area 2 (Zα2) eluting between 41 and 48 min (Fig. 2) to quantify the changes after extrusion. ANOVA results showed that the ratio of extrudates to grits of α-zeins were always less in chromatograms of harder grit extrudates than in softer grit extrudates, indicating less solubility of non-reduced zeins, possibly from greater protein aggregation in harder grits than softer grits during extrusion. The more hydrophobic non-reduced zein fractions appeared to be more affected by extrusion since the Zα2 peaks markedly changed and decreased after extrusion. Thus, the specific groups of proteins in Zα2 seem to have a specific role in the extrusion of maize. More research is needed on this group of proteins to determine their role in extrusion processing and quality of maize.

Extrusion processing

Maize kernel hardness and screw speed of extruder significantly influenced specific mechanical energy (SME) and physical properties of extrudates (Table V). However, their interaction did not have a significant impact on such response variables. The simple correlation coefficients and their significance for some physical properties of extrudates and extrusion processing parameters are shown in Table VI.

The SME required to produce an extrudate was lower in harder grits than in softer grits. This was more pronounced as screw speed increased. It is surmised that harder grits become more fluid earlier in the extruder and require less energy in the remainder of the extruder, resulting in lower die pressures. The increased SME with screw speed may attribute to greater effect of screw speed than that of melt viscosity during extrusion.

Expansion ratio (ER), one of the important physical properties of extrudate, was significantly higher in softer grit extrudates than harder grit extrudates ($P < 0.01$) (Fig. 3). The extrusion conditions such as die temperature and die pressure did not appear to be a main factor to explain the relationship between ER and screw speed. Although the total starch and amylose contents of softer grits were associated with ER of extrudates, the small differences of amylose content and total starch content between hard and soft grit extrudates were not likely to fully explain the distinctive difference in ER between them (Table VII). Protein denaturation is followed by the aggregation of unfolded protein molecules during extrusion, consequently decreasing protein solubility. ANOVA showed that the ratios of α-zein extracted from extrudates to grits (under reducing and non-reducing conditions) were significantly lower in harder grits than in softer grits ($P < 0.05$). This indicates that zein proteins from softer grits aggregated less than those from harder grits. Therefore, the lower expansion in harder grit extrudates in this study may be attributed to the higher degree of protein aggregation compared to softer grit extrudates.

Water absorption index (WAI) of extrudates increased with screw speed to a maximum value at 300 rpm, and then decreased with further increases in screw speed except for T-2914 (Fig. 4). Particularly, hybrid AD776W had a significantly lower WAI compared to the others. ANOVA results indicated the availability of hydrophobic zeins might partially contribute to the WAI of final extrudates. In addition, the compact structure of extrudates resulting from the partial fragmentation of macromolecules appears to affect WAI, presumably by limiting water penetration. Water solubility index (WSI) of extrudates was distinctively higher in softer grit extrudates (Fig 5). A significantly positive correlation between WSI and ER was also found. This indicates that the larger ER provides a larger surface area for water to interact with starch and other soluble components. Oil absorption capacity (OAC) was significantly lower in harder grit extrudates, and was more pronounced at higher screw speeds (Fig. 6). Contrary to WAI, nonpolar side chains of proteins are believed to bind the hydrophobic chains of fat contributing to a higher oil absorption. It is surmised that protein molecules in softer grit extrudates are less aggregated and associated through chemical interactions and thus more easily fragmented into smaller molecules based on the results obtained from RP-HPLC and the physical property tests of extrudates, consequently making protein hydrophobic sites more accessible and thus proteins able to bind more oil molecules in softer grit extrudates. Our results may also support an oil entrapment mechanism because extrudates maximally absorbed oil in highly expanded extrudates, presumably due to greater number of micropores and higher surface area. Breaking stress (BS) of harder grit extrudates was higher than that of softer grits. BS of extrudates seems to be associated with the rates of protein aggregation and denaturation, cell size, and cell wall thickness.

Grit and extrudates free sulfhydryl, disulfide, and total sulfhydryl content

Maize kernel hardness significantly influenced both SS and TS contents per mg of protein, and SH content in total protein of extrudates, whereas the screw speed and maize kernel hardness x screw speed interaction did not exhibit a significant effect on SH, SS, and TS contents both per mg of protein and in total protein, implying that the shear force determined by screw speed had less influence on these contents. Table VIII shows free sulfhydryl (SH), disulfide (SS), and total cysteine (TS) contents per mg of protein and in total protein for maize grits and extrudates. SH contents in extrudates were lower than those in their corresponding grits. The decrease in SH content may be attributed to the denaturation and aggregation of protein molecules through disulfide formation and hydrophobic interaction after extrusion. This result is consistent with that by RP-HPLC where decreased protein solubility likely occurred due to protein denaturation and aggregation. The decrease in SH content of extrudates coincided with an increase in disulfide content. The increase in SH content with a decrease in SS content might be related to the cleavage of disulfide bond through a combination of shear force and high temperatures during extrusion. However, the thiol-disulfide interchange reaction unlikely occurs during extrusion processing and seems to have a minor effect since it generally requires longer mixing times and sufficient water. TS contents of extrudates were also lower in extrudates than those of their corresponding grits. In this study, no significant differences in amounts of SH, SS, and TS between softer and harder grits/extrudates were found, implying that these differences by themselves may not significantly influence the physical and textural properties of final extrudates.

The results obtained from this study suggest that protein molecules in hard and soft grit extrudates have similar level of disulfide bond composition, though the proteins in soft grit extrudates interact less through hydrophobic bonds during extrusion. As a result, proteins in soft grits more easily dissociated resulting in increases in protein solubility. Therefore, the major chemical interaction to create the differences in the physical and mechanical properties of extrudates between hard and soft grit extrudates seems to be the hydrophobic interactions of protein molecules rather than disulfide bond formation under extrusion conditions used for this study. However, more research into the effects of other extrusion processing variables is needed to provide a better insight of the effect of maize endosperm texture and the role of biochemical actions on the quality of extrudates.

Practical Impacts of Research

a. Short Term Impacts

Prediction of end-use performances of maize has not been very practical by estimating several attributes commonly used in processing industry since maize samples have diverse environmental and genetic variability. As an outcome of this project, we created the decision structure through integrating and evaluating the physical, structural, spectral, and biochemical properties of reference maize samples with environmental and genetic diversity. This enables us to select and assign maize samples best suitable for producers, intermediate processors, and end-use processors. The decision structure may be strengthened by adding new maize samples produced in either environmentally diverse areas or across years into existing pool of maize.

b. Long Term Impacts

Unpredictable end-user performances and inconsistent results of testing methods are not a dilemma limited in maize industry but also other grains' since they have unique physical, structural, and biochemical properties modified by environmental and genetic conditions. This requires many measurements of quality attributes to characterize grains for the utilization. The best decision structures may be developed by evaluating many quality attributes of grains using the procedure as described in this study. This will help the grain industry to manage grain quality and reduce the risk of selecting grain samples with poor quality for a specific processing.

List of Publications Resulted from the Research

Lee, K.M., Herrman, T.J., Lingenfelser , J., and Jackson, D. S. 2005. Classification and prediction of maize hardness-associated properties by using multivariate statistical analyses. J. Cereal Sci. 41:85-93.

Lee, K.M. Bean, S.R. Alavi, S., and Herrman, T.J. 2005. Physical and biochemical properties of maize hardness and extrudates with particular focus on protein characteristics. J. Cereal Sci. (*Submitted*)

List of Reports to Other Sponsors

Kansas Corn Commission
Anderson Endowment administered through the Ohio State University

Table I

Correlation Variables with More Than 0.45 of Absolute Correlation in 3 Rotated Factors and Their Correlations [a]

Factor	**Variables and their correlation**
Factor 1	TW (0.77), DEN (0.83), TTG (0.58), DEN_P (0.79),TADD (0.64), W860 (-0.71)
Factor 2	STARCH (0.86), OIL (-0.86)
Factor 3	PRO (0.95), STARCH (-0.48)

[a] **DEN** = NIT density; **DEN_P** = air pycnometer density; **OIL** = oil content; **PRO** = protein content; **TADD** = Tangential Abrasive Dehulling Device test; **TTG** = time to grind in Stenvert Hardness Tester; **TW** = test weight; **STARCH** = starch content; **W860** = the absorbance at 860 nm in NIT.

Table II

The Means and Standard Deviations of Proximate Constituents
and Hardness-Associated Properties for Each Cluster [a]

Cluster	**No. of obs.**	**Protein (%)**	**Oil (%)**	**Starch (%)**	**TW (lb/bu)**	**DEN (g/cm^3)**	**DEN_P (g/cm^3)**	**TADD (%)**	**TTG (sec)**	**W860**
1	82	8.91±0.419a[*]	3.68±0.252a	60.17±0.443a	59.7±1.074a	1.293±0.007a	1.29±0.010	40.4±4.284a	21.75±1.949a	2.657±0.088ac
2	29	8.12±0.503de	3.36±0.280de	61.13±0.346b	58.5±1.156	1.283±0.014a	1.272±0.013cd	35.8±5.453bc	21.49±2.164a	2.707±0.100bc
3	20	8.64±0.351ab	3.31±0.249ab	60.85±0.289b	59.2±1.513abc	1.299±0.010bd	1.287±0.015ab	42.0±3.530	24.21±1.982b	2.589±0.082ae
4	36	8.20±0.342cd	3.00±0.204cd	61.73±0.391c	59.4±1.137abc	1.300±0.009bd	1.278±0.010bc	37.4±3.028bc	23.97±2.420b	2.643±0.077ac
5	21	8.82±0.326ab	3.76±0.212ab	60.00±0.306a	58.8±0.836bc	1.280±0.008a	1.281±0.013abc	38.3±3.793ac	19.35±1.631c	2.737±0.085b
6	12	7.85±0.578	3.28±0.192	60.84±0.624b	56.8±1.266d	1.255±0.009c	1.251±0.010	30.8±2.807f	18.15±2.396c	2.827±0.120d
7	14	7.91±0.250de	3.62±0.218de	60.22±0.361a	55.6±0.813	1.247±0.015c	1.231±0.008	27.8±3.970f	17.67±1.870c	2.997±0.097
8	25	8.52±0.576bc	3.65±0.221bc	59.97±0.521a	57.2±1.217d	1.265±0.013d	1.262±0.020	34.3±4.279b	19.30±1.987c	2.828±0.097d
9	3	8.00±0.350de	3.18±0.029de	61.87±0.176c	61.6±1.353	1.312±0.003e	1.304±0.013e	44.4±4.779	28.17±4.015d	2.541±0.107e
10	6	9.30± 0.527	3.72±0.191	59.93±0.376a	60.1±1.606a	1.307±0.010de	1.311±0.009e	43.6±3.788	27.35±4.149d	2.545±0.094e
LSD		0.3525	0.1936	0.3419	0.9388	0.081	0.0099	3.868	1.7581	0.0744

[a] Data shown are mean ± standard deviation of number of observations within each cluster. Data followed by the same letter are not significantly different at $p<0.05$.

Table III

The Clusters with the First-and Second-Largest Number of Observations
and Their Average Posterior Probabilities Resulting From Cross-Validation Method

From cluster	Total obs.	First classified cluster			Second classified cluster		
		Cluster	Obs.[a]	Post_P [b]	Cluster [c]	Obs.[d]	Post_P
1	82	1	80	0.925	3	2	0.605
2	29	2	25	0.762	1	2	0.526
3	20	3	16	0.778	4	2	0.761
4	36	4	35	0.911	2	1	0.711
5	21	5	17	0.777	1	3	0.682
6	12	6	11	0.962	2	1	0.440
7	14	7	13	0.999	6	1	0.703
8	25	8	23	0.975	2	2	0.483
9	3	9	3	0.999	.	.	.
10	6	10	4	0.837	1	2	0.887

[a] The number of observations classified correctly by discriminant rule
[b] Posterior probability
[c] The cluster number into which the observations misclassified
[d] The number of observations misclassified by discriminant rule

TABLE IV

Correlation Coefficients (r) between Hardness-Associated Properties and Contents of Endosperm Protein, Total Zein and Zein Subclasses [a]

	Protein	**Total zein**	**α-zein**	**β-zein**	**γ-zein**
TW	0.925**	0.917** (0.383)[b]	0.882* (0.311)	- 0.460 (-0.208)	0.581 (0.209)
DEN_P	0.841*	0.669 (-0.624)	0.662 (-0.385)	-0.396 (-0.088)	0.353 (-0.258)
TADD	0.951*	0.894* (0.031)	0.861* (0.058)	-0.470 (-0.256)	0.584 (0.212)
TTG	0.187	0.228 (0.153)	0.418 (0.577)	-0.875* (-0.894*)	-0.443 (-0.671)
W860	-0.900*	-0.879* (-0.235)	-0.928** (0.628)	0.743 (0.922*)	-0.291 (0.583)

[a] **DEN_P** = air pycnometer density; **TADD** = Tangential Abrasive Dehulling Device test; **TTG** = time to grind in Stenvert Hardness Tester; **TW** = test weight; **W860** = the absorbance at 860 nm in NIT.

[b] Partial correlation coefficients at constant protein content.

** = Significance at 1% level.

* = Significance at 5% level.

TABLE V
Statistical Significance (P-value) for the Effects of Maize Kernel Hardness and Screw Speed on SME, ER, WAI, WSI, OAC, and BS [a]

	SME	**ER**	**WAI**	**WSI**	**OAC**	**BS**
Hardness (H)	.048	<.01	.091	<.01	.01	<.01
Screw Speed (S)	<.01	<.01	.095	<.01	<.01	<.01
H x S	.575	.288	.949	.547	.056	.434

[a] SME = specific mechanical energy (kJ/kg); ER = expansion ratio; WAI = water absorption index (g water/g solid); WSI = waster solubility index (g solid/g water); OAC = oil absorption capacity (g oil/g solid); BS = breaking stress (kPa).

TABLE VI

Simple Correlation Coefficients (r) and Their Significance (P – value) for the Physical Properties of Extrudates and Extrusion Processing Conditions [a, b]

	RPM	**Die_T**	**Die_P**	**ER**	**PD**	**SME**	**WAI**	**WSI**	**OAC**
Die_T	0.435 (.071)								
Die_P	-0.612 (<.01)	-0.133 (.598)							
ER	0.843 (<.01)	0.430 (.075)	-0.356 (.147)						
PD	-0.866 (<.01)	-0.447 (.063)	0.365 (.137)	-0.984 (<.01)					
SME	0.904 (<.01)	0.538 (.021)	-0.364 (.138)	0.858 (<.01)	-0.853 (<.01)				
WAI	0.407 (.094)	-0.191 (.448)	0.035 (.889)	0.622 (<.01)	-0.612 (<.01)	0.396 (.104)			
WSI	0.820 (<.01)	0.553 (.017)	-0.242 (.334)	0.938 (<.01)	-0.950 (<.01)	0.864 (<.01)	0.553 (.017)		
OAC	0.824 (<.01)	0.474 (.047)	-0.254 (.309)	0.783 (<.01)	-0.814 (<.01)	0.884 (<.01)	0.381 (.119)	-0.894 (<.01)	
BS	-0.848 (<.01)	-0.415 (.087)	0.412 (.089)	-0.910 (<.01)	0.922 (<.01)	-0.853 (<.01)	-0.524 (.026)	-0.865 (<.01)	-0.841 (<.01)

[a] **BS** = breaking stress (kPa); **Die_P** = die pressure (psig); **Die_T** = die temperature (°C); **ER** = expansion ratio; **OAC** = oil absorption capacity (g oil/g solid); **PD** = piece density (g/cm^3); **RPM** = screw speed (rpm); **SME** = specific mechanical energy (kJ/kg); **WAI** = water absorption index (g water/g solid); **WSI** = waster solubility index (g solid/g water).

[b] Parenthesis below correlation coefficient indicate significant level (P - value).

TABLE VII

Total Starch and Amylose Contents (%), and
the Ratio of Amylose and Amylopectin of Grits Used for Extrusion [a]

Hybrids	**Starch (%, wb)**	**Amylose (%)**	**AM/AP [b]**
AD776W*	74.72±1.08	25.25±1.96	0.338±0.036
AD792W*	75.13±0.81	25.51±0.92	0.343±0.017
T-2914*	74.96±0.89	25.39±1.04	0.341±0.019
P61-491	76.05±0.81	24.10±0.57	0.318±0.010
NK70-D5	75.92±1.02	24.82±0.69	0.330±0.012
A6887	76.23±0.92	24.19±1.60	0.319±0.028

[a] Asterisk indicates harder hybrid.
[b] **AM** = amylose; **AP** = amylopectin.
[c] The values shown are mean ± standard deviation of four replicates.

TABLE VIII

The Free Sulfhydryl, Disulfide, and Total Sulfhydryl Contents
Per mg of Protein and in Total Protein in Maize Grit and Extrudates

Hybrids[a]	**Screw speed (rpm)**	**Protein (mg)**	***Free sulfhydryl***		***Disulfide***		***Total cysteine***	
			mg of protein[b]	***Total protein***[c]	***mg of protein***	***Total protein***	***mg of protein***	***Total protein***
AD776W (*)	Grit	8.73	41.1 a	358.7 a	28.5 cdef	248.9 defg	98.1 b	856.5 ab
	200	8.49	32.5 bcd	275.8 bcdef	23.4 hij	198.5ij	79.3 fg	672.7 ghi
	300	8.65	33.2 bc	287.4 bc	24.5 ghij	211.4 hij	82.1 efg	710.2 efgh
	400	8.82	31.5 cd	277.9 bcde	21.8 j	197.8 j	76.4 gh	673.4 ghi
AD792W (*)	Grit	9.51	30.0 cdef	285.5 bc	31.4 bcde	298.3 abc	92.8 bcd	882.0 ab
	200	9.03	25.9 g	234.1 hijk	29.4 cdef	265.2 cd	84.7 ef	764.6 cdef
	300	9.26	27.2 efg	251.8 efgh	27.9 defg	258.3 def	83.0 efg	768.3 cdef
	400	9.42	24.8 g	233.7 hijk	28.9 cdef	272.4 bcd	82.7 efg	778.5 cd
T-2914 (*)	Grit	8.92	31.8 cd[d]	283.6 bcd	33.7 ab	300.1 ab	99.1 b	883.9 ab
	200	8.75	30.4 cde	265.7 cdefg	27.5 efg	240.9 defgh	85.4 def	747.3 def
	300	8.79	24.2 g	212.8 jk	22.4 ij	198.9 ij	69.5 h	610.5 i
	400	8.85	26.5 gf	234.5 hijk	28.4 cdef	250.8 defg	83.2 efg	736.1 defg
P61-491	Grit	8.26	36.0 b	297.5 b	37.3 a	307.8 a	110.5 a	913.2 a
	200	8.20	29.6 def	242.3 ghij	32.0 bc	261.9 de	93.5 bc	766.1 cdef
	300	8.26	29.9 cdef	246.5 fghi	31.4 bcd	259.6 def	92.7 bcd	765.65 cdef
	400	8.38	30.2 cde	253.3 defgh	313 bcde	261.9 de	92.8 bcd	777.0 cde
NK70-D5	Grit	8.51	33.1 bcd	281.8 bcde	31.9 bc	271.2 bcd	96.9 b	824.2 bc
	200	8.42	25.8 g	217.2 ijk	26.5 fghi	222.7 ghij	78.7 fg	662.6 hi
	300	8.48	25.1 g	213.1 jk	27.1 fgh	229.6 efghij	79.3 fg	672.3 ghi
	400	8.81	24.5 g	215.7 ijk	27.8 defg	245.2 defg	80.2 efg	706.1 fgh
A6887	Grit	7.84	32.3 cd	252.9 defgh	33.5 ab	262.8 de	99.4 b	778.5 cd
	200	7.67	27.4 efg	210.1 k	30.0 bcdef	230.0 efghij	87.3 cde	670.1 ghi
	300	7.85	26.9 efg	211.6 jk	29.5 cdef	231.7 efghi	86.0 cdef	674.9 ghi
	400	7.92	27.3 efg	216.5 ijk	28.7 cdef	227.3 fghij	84.7 ef	671.2 ghi

[a] Asterisk indicates harder hybrid.

[b] The values are mean of duplicate measurements and reported in nmol/mg of protein.

[c] The values are calculated by protein content (mg) x contents (nmol) per mg of protein, reported in nmol.

[d] The data followed by the same letter are not significantly different at $P < 0.05$.

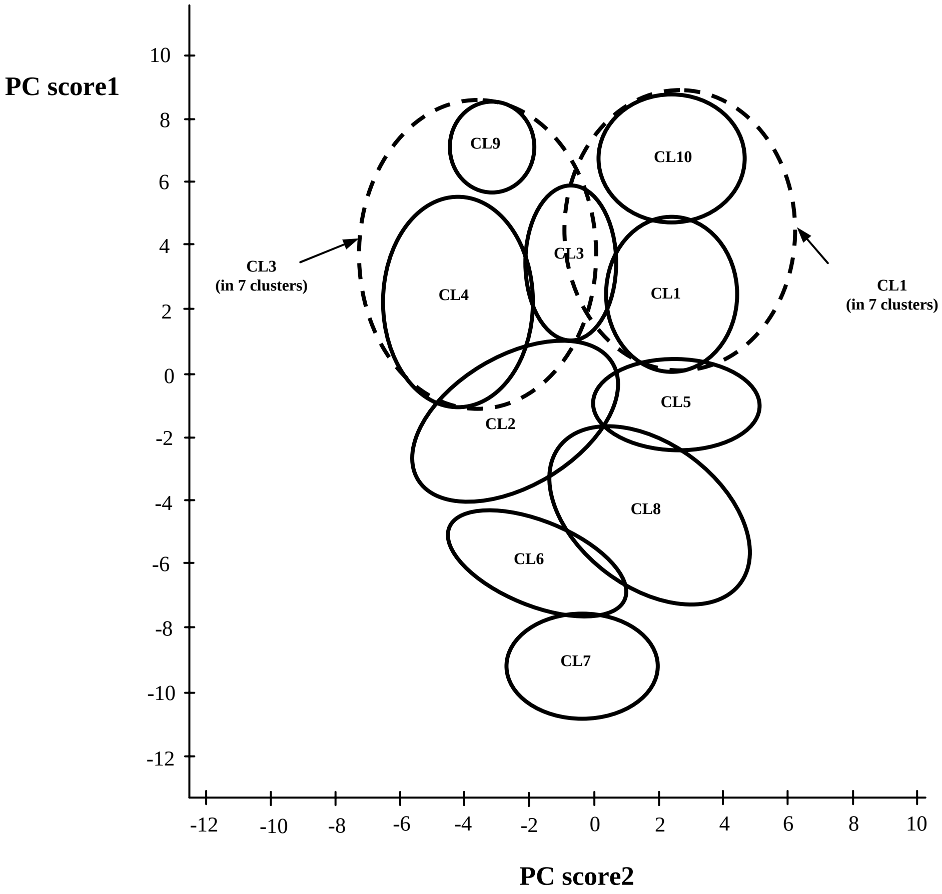

Fig. 1. The plot created by the first two principal component (PC) scores; Solid lines indicate the clusters in total 10 clusters and dot lines in 7 clusters (Clusters 2,5,6,7 and 8 are common in both clusters)

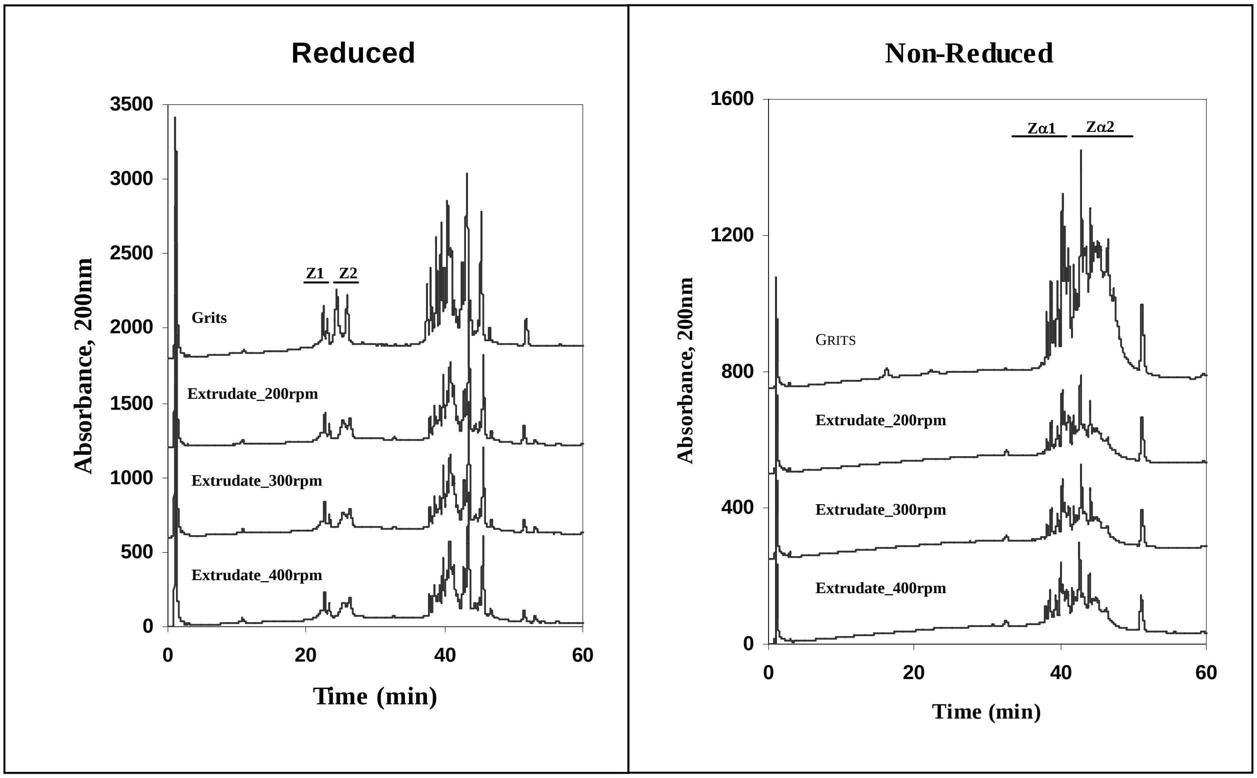

Fig. 2. RP-HPLC chromatograms of alcohol extractable proteins in maize grits and extrudates at different screw speeds using t-butanol with or without reducing agent (β-ME)

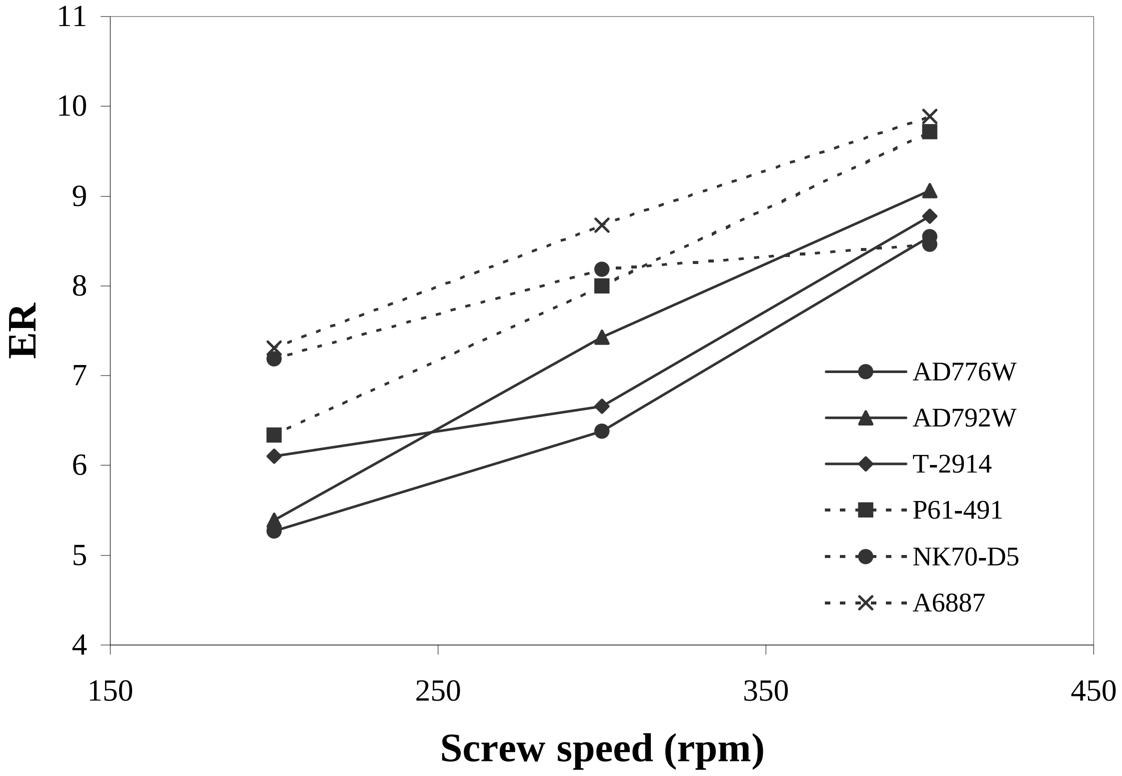

Fig. 3. The effect of maize kernel hardness and screw speed (rpm) on expansion ratio (ER) of extrudates. Solid lines represent harder grit extrudates and dotted lines softer grit extrudates, respectively.

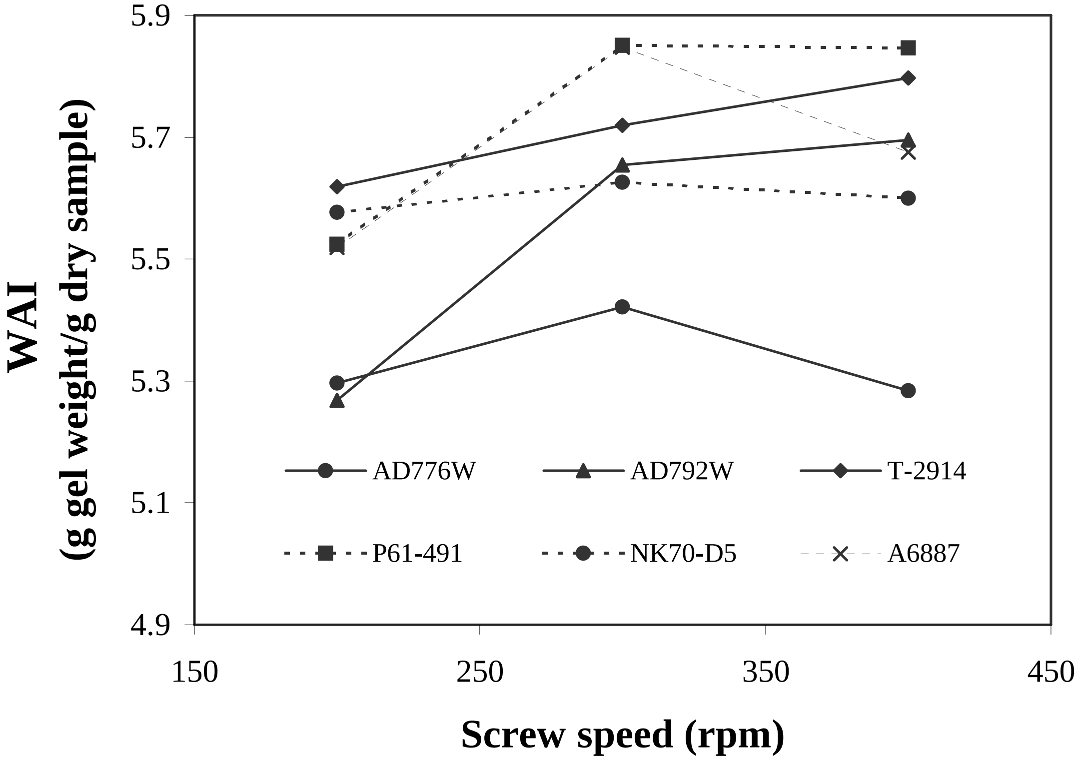

Fig. 4. The effect of maize kernel hardness and screw speed (rpm) on water absorption index (WAI) of extrudates. Solid lines represent harder grit extrudates and dotted lines softer grit extrudates, respectively.

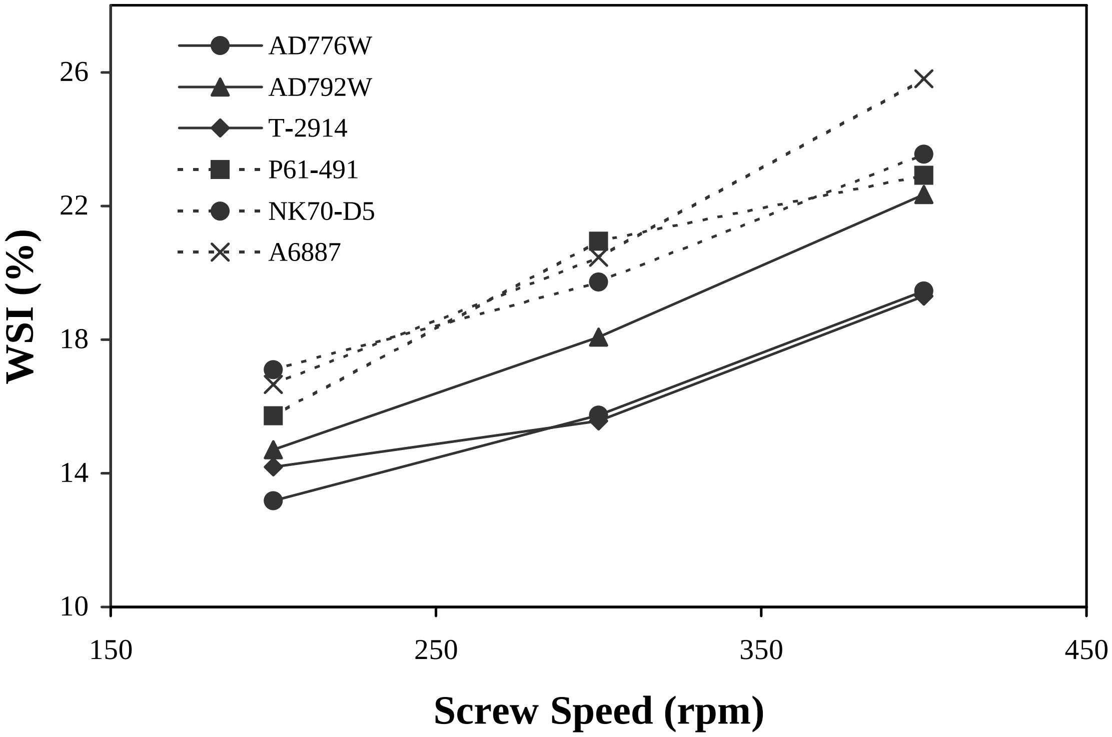

Fig. 5. The effect of maize kernel hardness and screw speed (rpm) on water solubility index (WSI) of extrudates. Solid lines represent harder grit extrudates and dotted lines softer grit extrudates, respectively.

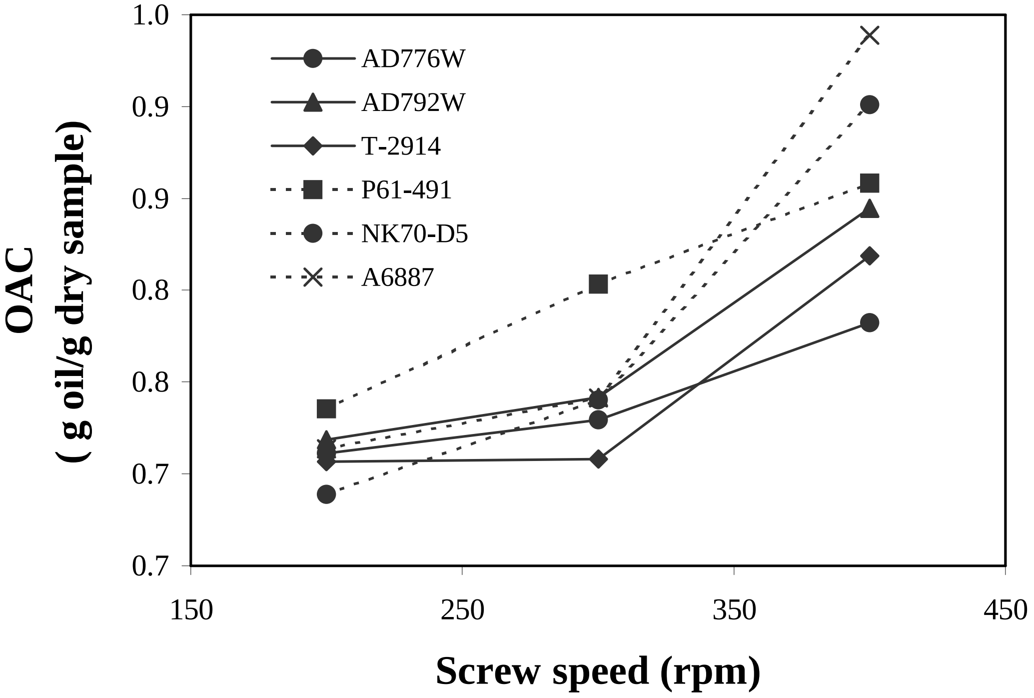

Fig. 6. The effect of maize kernel hardness and screw speed (rpm) on oil absorption capacity (OAC) of extrudates. Solid lines represent harder grit extrudates and dotted lines softer grit extrudates, respectively.