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Sequential extraction processing: alternate technology for corn wet milling

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Sequential extraction processing: Alternate technology for corn wet milling

by

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DEDICATION

This thesis is lovingly dedicated to my husband, Rok, whose love and unwavering support enabled me to realize my ambitions and brought me boundless happiness. I am more than proud to share with him the honor that this work brings.

INTRODUCTION

Wet Corn Milling

The bulk of processed corn in the United States undergoes wet milling. The process involves an initial water soak under carefully controlled conditions of temperature, time, sulfur dioxide concentration and lactic acid content to soften the kernels and facilitate separation of the components. The corn is then milled and its constituents are separated by screening, centrifuging and washing to produce starch, oil, and feed by-products such as protein (gluten) and fiber (Figure 1). The cornstarch is used in the manufacture of sweeteners and for fermentation into industrial solvents such as ethanol, butanol, isopropanol and acetone. Ethanol is also utilized as a fuel extender.

Wet milling techniques are preferred to dry milling because the starch is recovered in greater yield and purity. However, wet milling is both capital- and energy-intensive. The process has remained largely unchanged over the past 50 years, but the increased demand for high-fructose corn syrups and fuel ethanol in recent years now dictate the need to adopt more cost-effective, less polluting measures to process corn into starch so that the industry can remain competitive and expand.

Sequential Extraction Processing of Corn

The Sequential Extraction Process (Figure 2) is a radical new approach to corn milling which hopes to reduce processing costs, increase yields of high-value products, and upgrade the value of by-products. Anticipated elements of the process are: a) the sequential extraction of crude oil using solvents which can be produced from cornstarch fermentation; b) the simultaneous dehydration of the solvent during oil extraction; c) use of aqueous alcohols to extract protein; d) enhancing extraction of proteins using either ultrasonics or homogenization; and e) recycling solvents from alcohol fermentation, particularly ethanol, to upstream steps of extraction and reduce the costs of drying

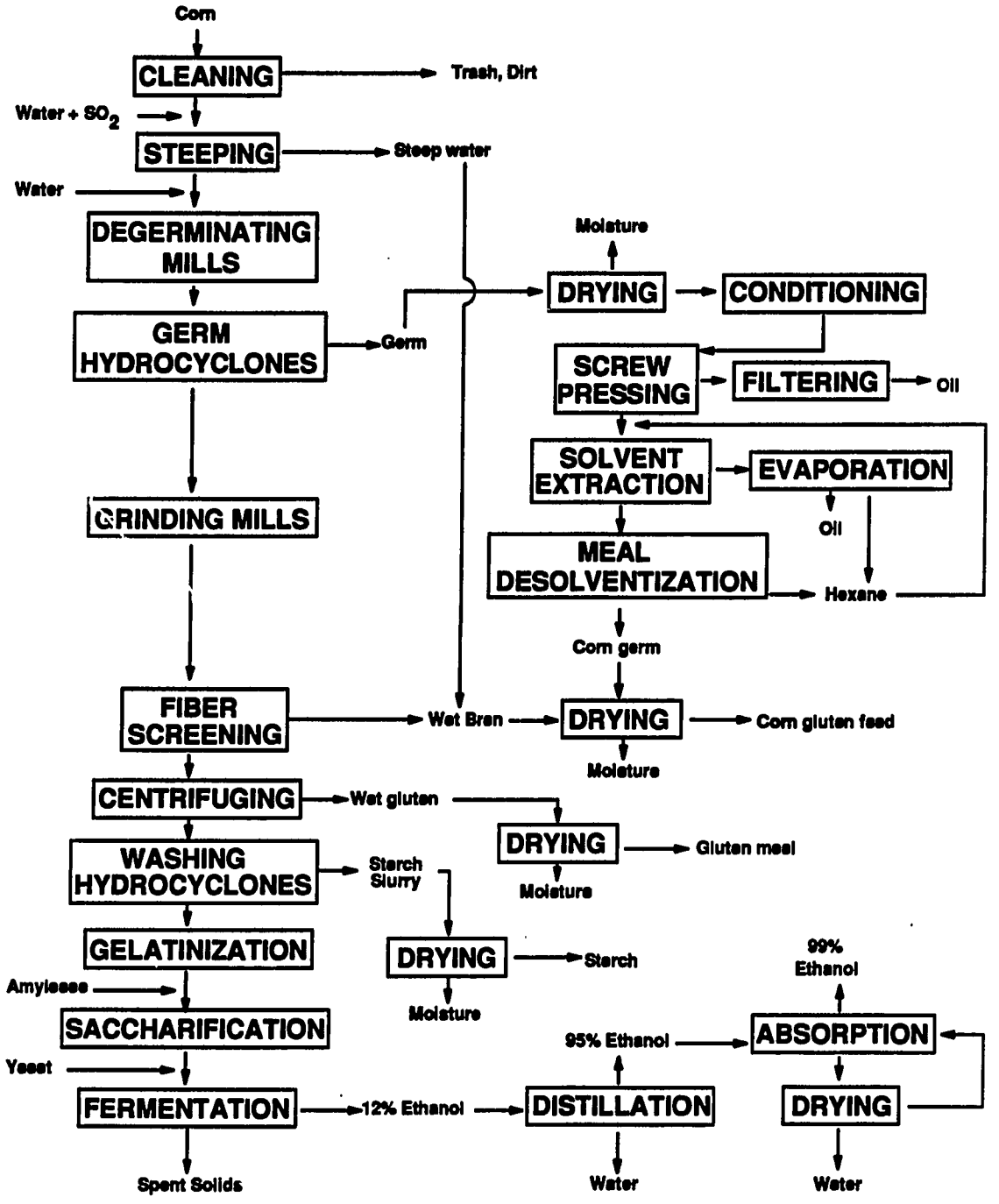


Figure 1. Conventional wet milling of corn

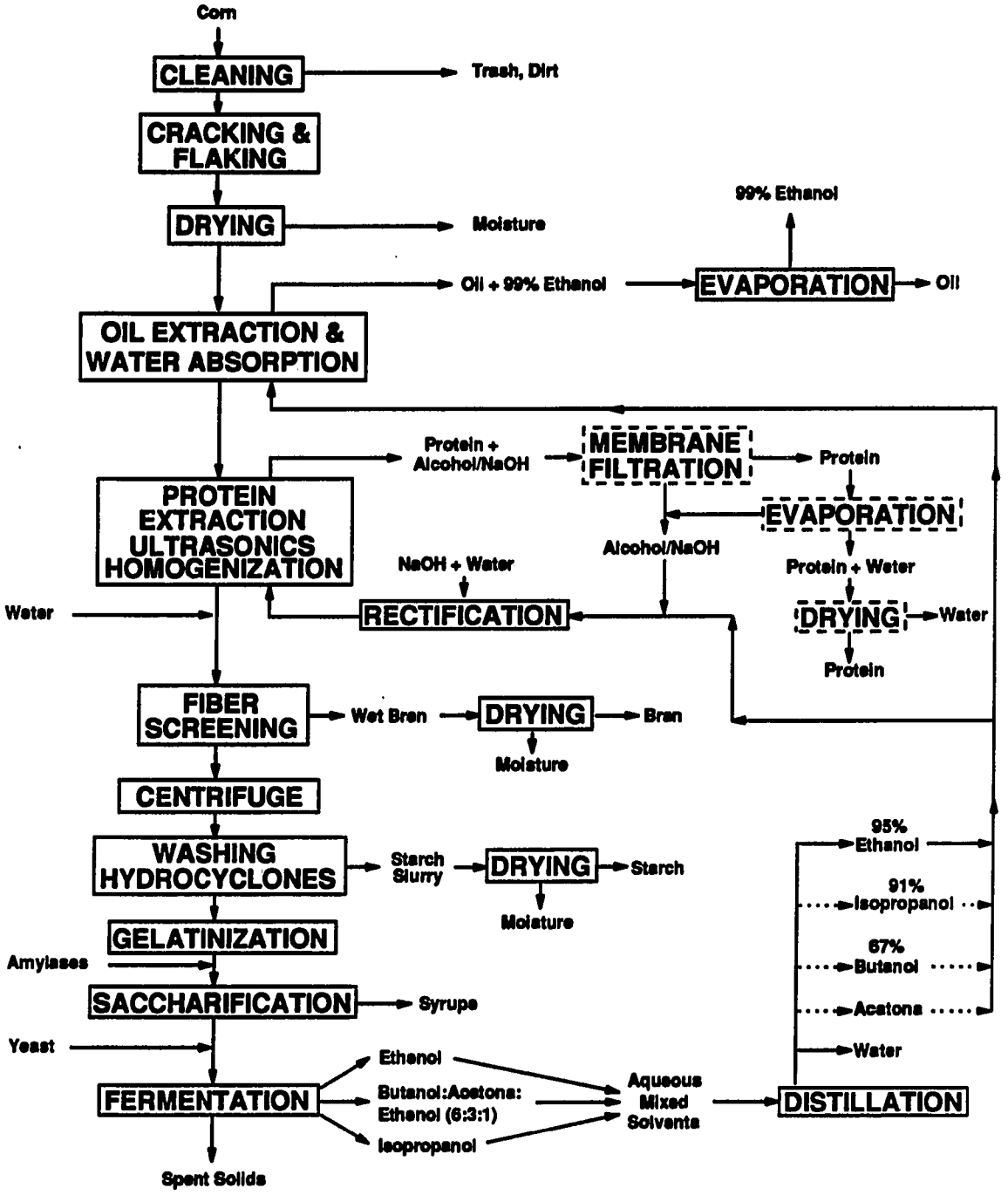


Figure 2. Sequential extraction milling of corn

alcohol. The feasibilities of applying the first four elements to dried, flaked whole corn were evaluated in the first four sections of this manuscript. The fifth and last part verified that all of the elements studied separately in the previous sections could be integrated into a single process.

Oil extraction using solvents from cornstarch fermentation

In their comprehensive review of alternative solvents for oilseeds extraction, Johnson and Lusas (1983) reported that ethanol and isopropanol have been used to commercially extract vegetable oils during periods of petroleum shortages. The solubility of vegetable oils in these alcohols varies greatly with temperature and water content of the alcohol (Figure 3). Oils are completely miscible in each anhydrous alcohol at its boiling point and only slightly soluble at ambient temperature. At lower alcohol concentrations, oil solubility is low even at the boiling point (Rao et al., 1955; Rao and Arnold, 1956a, 1956b). Beckel et al. (1948a, 1948b) developed a non-distillation extraction process using aqueous ethanol to recover soybean oil. Karnofsky (1981) and Hassanen et al. (1985) recently developed sequential extraction processes using ethanol to extract oil and aflatoxin from cottonseed. Harris et al. (1947, 1949) investigated the potential of isopropanol as a solvent for cottonseed extraction and developed a pilot plant process which also removes gossypol from cottonseed. In 1961, Vaccarino and Vaccarino described an industrial acetone extraction process for cottonseed which produced oil of comparable quality to hexane-extracted cottonseed oil and gossypol-free cottonseed meal. Butanol has been used to extract lipids from corn germ and endosperm (Weber, 1978) but Hron et al. (1982) contend that butanol cannot be considered seriously because of its toxicity and its high boiling point (over 93^{o}C) which results in excessive energy for recovery and increased refining loss for cottonseed oil.

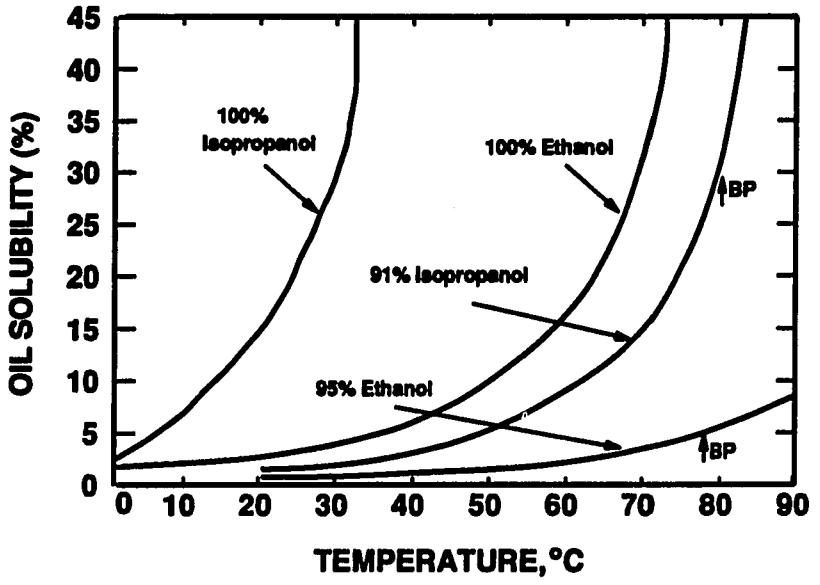


Figure 3. Solubilities of cottonseed oil in alcohols

Alcohol dehydration

Studies on alcohol dehydration have focused on ethanol only. Ladisch et al. (1984) designed a pilot-scale adsorber which used cornmeal to dehydrate ethanol vapors. Other biomass materials which have been screened for ethanol dehydration potential were cellulose, xylan, corn and potato starches, corn residue, and bagasse (Hong et al., 1982). Chien et al. (1988) reported on a column extraction process which simultaneously extracted oil from ground corn and dehydrated 95% ethanol at 68^{0}C.

Ladisch and Tsao (1982) developed a non-distillation process for the energy efficient recovery of anhydrous ethanol. The method involves partial distillation of 12% ethanol, a product of crude fermentation, to a 70-90% aqueous product followed by water absorption using cellulose, corn residue or cracked corn.

Extraction of corn proteins

Zein and glutelins are the major proteins in the corn endosperm. Zein is the alcohol-soluble fraction while glutelins are soluble in dilute alkali solutions (Osborne and Mendel, 1914). Together, they comprise almost 80% of the grain nitrogen (Landry and Moureaux, 1970). Albumins (water-soluble proteins) and globulins (soluble in dilute salt solutions) are minor fractions in the endosperm but they constitute 28% and 24%, respectively, of the germ proteins (Paulis and Wall, 1969). Most of the studies on corn protein extraction have focused on the prolamins (zein) and glutelins. Russell (1980) reported that 97% of the total zein in dry-milled corn endosperm can be solubilized by using 55-65% (w/w) ethanol at solvent:endosperm ratios of 20 ml:1 cm^3. Increasing NaOH concentrations, extraction temperatures, and solvent:endosperm ratios promoted the solubilization of glutelins. They also achieved nearly 90% solubilization of the total protein in corn endosperm by employing two-step sequential extractions of zein and glutelins. Lawhon (1986) claimed that food grade protein can be obtained from corn by using a process which involves extracting the protein with alkali or alkali/alcohol solutions, either with or without sonication, and recovering the protein from the extract by ultrafiltration. The total protein recovery was about 74% for undegermed corn meal and 65% from degerned corn meal using the mixture 55% ethanol:45% 0.1 N NaOH at 40-45^{0}C and a solvent:meal ratio of 25:1. Concon (1973) reported that 97% of the zein can be recovered if NaOH is added after pre-solubilization of the protein in 70% ethanol.

Albumins and globulins must also be considered in the extraction in order to produce high-quality starch and maximize by-product return. A German group has reported that homogenization can be incorporated into conventional wet milling to improve protein-starch separation and to reduce steeping times (Huster et al., 1983; Meuser and German, 1984). Increased protein yields were observed with the use of sonication (Lawhon, 1986).

Advantages of Sequential Extraction Processing

If sequential extraction processing of corn is shown to be practical, several advantages over conventional wet milling are likely to result. Since steeping will no longer be employed, adverse effects of SO_2 would be eliminated, thus improving the quality of the protein by-products and reducing potential health hazards from sulfites. The protein product would be food-grade zein-rich fraction which is expected to be useful as food protein ingredient in applications different from those of soy proteins. Sequential extraction should easily be converted into a continuous operation, thereby eliminating capital requirements for expensive batch steeping facilities and attendant waste disposal problems. The number of milling steps would be reduced. Since the oil will be extracted as part of the milling process, losses in oil yield and quality due to transporting of corn germ from the mill to the crushing plant will be eliminated. Screw presses for oil recovery, which are expensive to purchase, operate, and maintain will not be needed. Thus, there is potential for major reductions in energy, water use, and capital investment. Such reductions could increase the fraction of the finished product value returned to farmers, make corn products more competitive in the market and, consequently, expand the markets for corn.

Research Objectives

The main objective of this study was to evaluate the feasibility of using solvents from cornstarch fermentation, particularly ethanol, to separate oil and protein from the starch and other corn components in a sequential extraction approach to corn milling. The specific objectives were: a) to assess the effects of various solvents and the extraction conditions on oil recovery from dried, flaked, whole corn; b) evaluate the feasibility of simultaneous alcohol dehydration and oil extraction; c) determine the effects of the various oil extraction solvents on the extraction (and/or denaturation) of corn protein fractions; d) establish optimum conditions for extraction and recovery of corn

protein; e) examine the potentials of sonication and homogenization to enhance protein yields; and, f) compare the yields of the recovered fractions to those obtained by traditional wet corn milling.

Explanation of Dissertation Format

The dissertation consists of five manuscripts which will be submitted for publication to professional journals and presents the results of original research conducted by the candidate under the guidance of her major professor. Literature cited in the Introduction of the thesis are presented in the section, "General References".

PART I. PERCOLATION EXTRACTION OF CORN OIL FROM WHOLE CORN AND ASSOCIATED PROTEIN LOSS

ABSTRACT

A laboratory extractor-simulator was developed to demonstrate the feasibility of extracting oil from undegermed corn, the first step in sequential extraction processing. The effects of flaking and grinding, corn variety, and extracting solvent, concentration and temperature on oil recovery were assessed. Protein loss during oil extraction was also evaluated.

Flaked corn showed better extraction characteristics than ground corn. Oil recovery was higher in varieties having substantial amounts of floury endosperm (soft dent and high-lysine corn). Ethanol, isopropanol, acetone, butanol, and the butanol:acetone:ethanol mixture (6:3:1) all showed oil recoveries which were either equal to or better than the 72% obtained by conventional prepress hexane extraction methods in industry. Greater oil recoveries were achieved using anhydrous concentrations and temperatures close to the boiling point of the solvent. Low temperature extraction, however, appears feasible when using butanol:acetone:ethanol, ethanol, and isopropanol. Butanol, isopropanol and ethanol reduced the total crude protein content of the flaked corn, particularly when high aqueous concentrations and high temperatures were used for oil extraction.

INTRODUCTION

Importance of Corn Oil

Corn is a cereal crop and as such has a relatively low oil content (4.5%, compared to 20% for soybeans). Corn oil is recovered as a by-product of corn milling and its production is highly dependent on the demand for the major corn products of corn meal, corn syrups, starch, and alcohol (Haumann, 1985).

Although corn oil is considered a minor oil in the edible vegetable oils market, it is probably the best known among U.S. consumers. Corn oil has the reputation of being a high-quality oil for a number of reasons. Foremost among these are the nutritional and health benefits given by its high concentration (60%) of polyunsaturated essential fatty acids which have been shown to have a positive role in lowering blood cholesterol. Its inherent antioxidants and low linolenic acid content impart good oxidative stability. The high degree of unsaturation of corn oil allows it to remain liquid even under refrigeration, a characteristic desired in salad oils. Its light delicate flavor and golden color further add to its appeal to consumers as a cooking oil (Reiners and Gooding, 1970).

Corn Oil Processing

Crude corn oil Both wet and dry corn millers separate the germ from the corn kernel and recovery of the germ represents about 80% of the total oil in the corn. Crude oil is obtained from the dried germ usually by a combination of mechanical expression and solvent extraction. Continuous screw expellers press the oil from the germ under high pressure and moderate heat. About 80% of the oil is recovered by pressing. The residual oil in the germ cake is obtained by extracting with hexane. The miscella is filtered and the solvent is removed by evaporation. The solvent from the germ cake and oil miscella is evaporated by heating and steam stripping, and is condensed for recycling.

Crude oil recovered by both methods is combined for further processing. Recovery by prepress solvent extraction is about 90% of the oil in the germ. Thus, total oil recovery from corn is about 72%.

Refined corn oil Crude corn oil undergoes refining to reduce or eliminate those components which diminish its quality. The oil is first degummed to remove most of the phospholipids and then treated with alkali to remove the free fatty acids, phospholipids and some color pigments. This is followed by bleaching to further remove pigments and residual phospholipids. The process is completed by deodorizing although hydrogenation may be done prior to this last step if used for margarine manufacture.

Alternatives for Corn Oil Extraction

Hexane costs have become a major factor in oil processing due to the 8-fold increase in its price over the past years (Johnson and Lusas, 1983). The scarcity of hexane in the early 1980s demonstrated the need for alternative solvents which are less dependent on petroleum for their sources (Hron et al., 1982). The high flammability of hexane, as well as, toxicological and environmental concerns regarding its use have further motivated the search for alternative solvents (Johnson and Lusas, 1983). Screw presses for oil recovery also add to production costs of oil recovery because they are expensive to purchase, operate, and maintain.

Solvents which are products of biomass fermentation have received considerable attention as possible alternatives to hexane because of their potential to be recycled for oil extraction. Saccharified cornstarch can be fermented by *Saccharomyces cerevisiae* to produce ethanol. Fermentation by *Clostridium acetobutylicum* produces an aqueous (80% water) mixture of butanol:acetone:ethanol (6:3:1). It is also possible to obtain only ethanol, butanol, or acetone with distillation of butanol:acetone:ethanol. Isopropanol is produced indirectly by reducing the acetone from the Weizmann fermentation process.

Alcohols Johnson and Lusas (1983) reported that ethanol and isopropanol have been used to commercially extract vegetable oils during periods of petroleum shortages. This was based on the early works of Beckel et al. (1948a, 1948b) on a non-distillation extraction process they developed to recover soybean oil. From 1955 to 1956, Rao et al. studied the solubilities of 13 common vegetable oils in aqueous ethanol. Rao and Arnold (1958) used a countercurrent pilot plant unit to extract oil from cottonseed flakes using aqueous ethanol. Their studies concluded that not only was the process feasible, it was also capable of yielding crude oil of prime quality and light colored meal of good quality with very little free gossypol content. Recently, Karnofsky (1981) and Hassanen et al. (1985) developed sequential extraction processes using ethanol to extract oil and aflatoxin from cottonseed.

Harris et al. (1947, 1949) were the first to investigate the potential of isopropanol as solvent for cottonseed oil extraction. Rao and Arnold (1957) determined the solubilities of several vegetable oils in aqueous isopropanol in experiments similar to their earlier ethanol studies. The solubility of oil increases during heating until the critical solution temperature is reached. The critical solution temperature of isopropanol also increases with moisture content and is about 82^{0}C for 91% isopropanol. Crude oil extracted with 91% isopropanol is superior to crude oil recovered by hexane, and is much lower in free fatty acid contents and phosphatides. Isopropanol/water mixtures were also effective in extracting aflatoxins from cottonseed. Youn and Wilpers (1981) developed the Shell Process which recovers oil from soybeans by countercurrent extraction using 91% isopropanol. The process has routinely achieved 0.3-0.7% residual oil in the meal.

Acetone Acetone was evaluated as a selective solvent for vegetable oils by Youngs and Sallans (1955) and in 1961, Vaccarino and Vaccarino described the elements of an industrial process which used acetone to extract oil from cottonseed. It was claimed that the process produced gossypol-free cottonseed meal, improved oil refining yields and produced oil of comparable quality to hexane-extracted cottonseed oil. It has

also been suggested that acetone in combination with hexane and water can be used to extract gossypol (Gastrock et al., 1965) and aflatoxin (Gardner et al., 1968). Hron and Kuk (1989) reported that cottonseed can be extracted with increased efficiency using acetone to produce meals containing low gossypol and without disagreeable catty odors.

Other solvents In her study of the corn germ and endosperm lipids, Weber (1978) reported that boiling water-saturated n-butanol extracted the most lipid from the endosperm and germ. She also emphasized that little attention has been given to the lipids in the endosperm even though these lipids may affect the properties and keeping quality of the milling fractions obtained from the endosperm.

Numerous other solvents with potential for oils extraction were presented in comprehensive reviews by Johnson and Lusas (1983) and Hron et al. (1982).

These solvents are also capable of solubilizing some of the proteins in the corn (Swallen, 1941), thus, it is expected that small amounts will be extracted with the oil. Since the proposed Sequential Extraction Process involves maximizing the recovery of the proteins after oil removal, it is therefore necessary to determine the degree of protein loss brought about by the oil extraction conditions.

Objectives of the Study

This research was undertaken to evaluate the feasibility of using solvents that could be produced by fermentation of cornstarch to extract oil from whole corn. Specifically, the study attempted to: determine the best method to prepare corn for extraction, determine factors affecting the efficiency of oil recovery, compare the yields of the recovered oil extracted by the various solvents and evaluate the effects of the oil extraction conditions (kind of solvent, concentration, and temperature) on the total protein content of the defatted corn.

MATERIALS AND METHODS

Corn Preparation Method

Dent corn, variety Pioneer 3732, was provided by the Agricultural Engineering Grain Quality Laboratory, Iowa State University. One batch of corn was cracked then flaked using a Roskamp rollermill (Model K, Roskamp Mfg., Inc., Waterloo, IA) while another batch was ground to various particle sizes using a Fitzpatrick hammermill (Model D, Fitzpatrick Co., Elmhurst, IL) and a Glenmills microhammermill IV (Glenmills Inc., Maywood, NJ). Both corn batches were dried to moisture contents of approximately 4% prior to extraction with 91% isopropanol at 65^{0}C.

Oil Extraction and Recovery

A laboratory extractor-simulator similar to that of Hassanen et al. (1985) was used to simulate percolation extraction and filtration extraction principles (Figure 1). The solvent was added to the corn at a ratio of 2:1 (w/w). This ratio was kept constant by weighing the miscella after every stage and using this weight as the amount of pre-heated fresh solvent to add to the corn in the next stage. Six stages were used at 10 min/stage followed by 5 min draining/stage.

Oil was extracted in duplicate runs from flaked undegermed corn with ethanol, isopropanol, acetone, butanol, and the mixture of butanol:acetone:ethanol (6:3:1) using two concentrations (aqueous and anhydrous) and two extraction temperatures per solvent (ambient temperature, except for ethanol where 40^{0}C was used, and the boiling point of the solvent). Percolation extraction with petroleum ether was also performed. The design of the experiment is given in Figure 2. The oil was recovered from the solvent by rotary evaporation. The oil was further separated from solid contaminants by washing with petroleum ether. The washings were filtered into a pre-weighed flask and the petroleum ether was allowed to evaporate using a rotary evaporator. Oil yields were

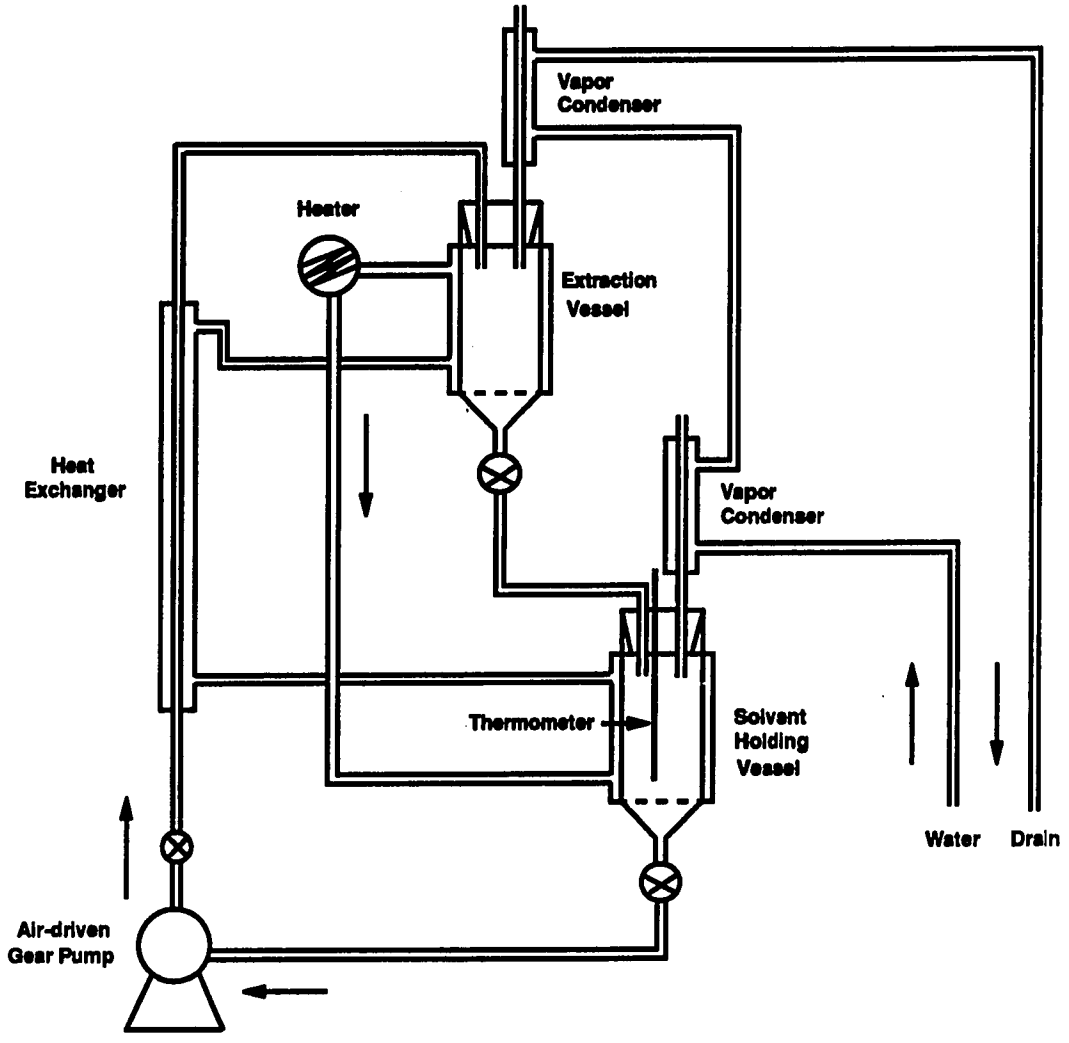


Figure 1. Schematic diagram of the laboratory extractor-simulator

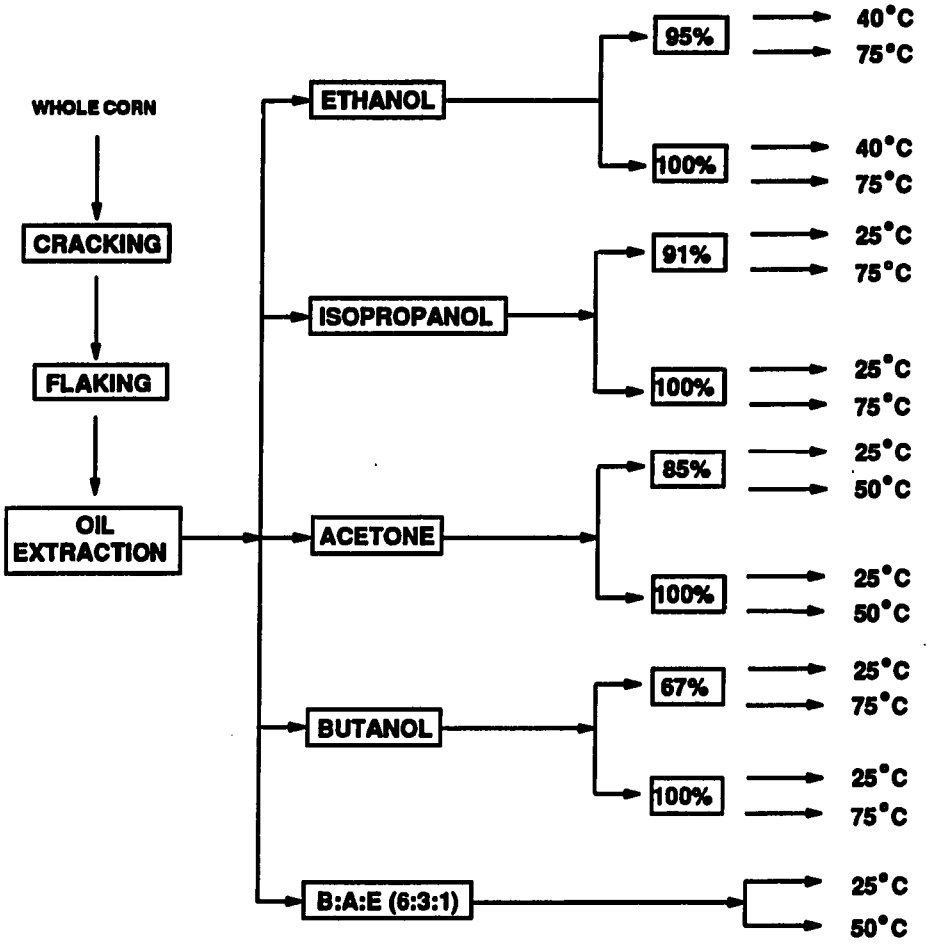


Figure 2. Experimental design for corn oil extraction

compared to determine which form or particle size gave a better extraction efficiency. The efficiency of extraction by each solvent was calculated and compared against conventional oil extraction.

The defatted flaked corn was air-dried and then vacuum-dried at 40°C. The dried samples were stored in sealed polyethylene bags for use in subsequent stages of the study while the recovered oils were stored in screw-capped vials for future analyses.

Varietal Effects on Oil Extraction

The effects of corn variety on oil extraction efficiency were also evaluated. Pioneer 3732 (medium-hard dent corn), Pioneer 3377 (soft dent corn, Pioneer Hi-Bred International Inc., Johnston, IA) and high-lysine corn (Crow's Hybrid Seed Co., Milford, IL) were extracted with 97.5% ethanol at 75°C using the laboratory simulator-extractor following the procedure described in the preceding section.

Chemical Analyses

Moisture, crude oil, and protein contents of the corn before and after oil extraction were determined by AACC standard procedures 44-15A, 30-20, and 46-13, respectively (AACC, 1983). Residues extracted with the oil were analyzed for protein content using MicroKjeldahl N determination (AACC, 1983). All determinations were performed in duplicate.

Statistical Analyses

Data were analyzed using a Statistical Analysis System (SAS, 1987) program. Significant differences among treatment means were identified using Duncan's Multiple Range Test or Least Significant Difference (LSD). The main and interaction effects were determined using the General Linear Models (GLM) procedure. Probability levels of $p \leq 0.05$ were considered significant.

RESULTS AND DISCUSSION

Corn Preparation Method

Relatively high amounts of oil could be recovered from both flaked and ground corn (Table 1). With grinding, higher oil recovery was obtained when smaller corn particles were used. This was probably due to greater surface area coming into contact with the solvent and greater cell distortion when the particle size was reduced. However, problems with fines were encountered with all ground samples. The bed of ground corn packed easily, reducing percolation of the solvent. No such problems were experienced with flaked corn, which gave the highest quantity of recovered crude oil. Flaking facilitates extraction by distorting cells and reducing the thickness of the corn particle, creating a shorter mass transfer distance (Norris, 1982).

Table 1. Effects of corn preparation method on oil extraction from Pioneer 3732 corn using 91% isopropanol at 65°C

Treatment			Residual[1] oil (% db)	Recovery[2] (%)
Preparation	Equipment	Size (mm)		
Flaking	Rollermill	0.25 (0.01 in)	0.30	93.8 ± 0.3[a]
Grinding	Fitzpatrick	2.38 (8 mesh)	0.68	86.2 ± 0.4[b]
	Hammermill	3.36 (6 mesh)	0.69	85.9 ± 0.6[b]
	Glenmills	1.54 (11 mesh)	0.77	84.3 ± 0.1[c]
	Micro-	2.00 (9 mesh)	0.87	82.2 ± 0.3[d]
	hammermill	4.00 (5 mesh)	1.12	77.0 ± 0.3[e]

[1]Initial oil content was 4.88% (db).

[2]Means with the same superscripts are not significantly different at $p \leq 0.05$.

Isopropanol also extracted other soluble, non-oil components from the corn which became visible as solid residues in the oil after the solvent was evaporated. The residue obtained from the ground corn contained 35-40% crude protein while the residue from the flaked corn had 44% crude protein (Table 2), but, since scant quantities of the solids were obtained, the amount of protein extracted with the oil was not significant. These preliminary experiments showed that flaking was the better method for preparing undegermed corn for oil extraction.

Table 2. Crude protein contents of residues extracted with oil from Pioneer 3732 corn

Treatment			Mean wt. residue (g)	Crude protein content[1] (% db)	Protein extracted (g/100 g dry corn)
Preparation	Equipment	Size (mm)			
Flaking	Rollermill	0.25 (0.01 in)	4.25	43.9 ± 0.3[a]	1.86
Grinding	Fitzpatrick	2.38 (8 mesh)	5.38	40.5 ± 0.0[b]	2.18
	Hammermill	3.36 (6 mesh)	3.82	41.5 ± 0.1[b]	1.58
	Glenmills	1.54 (11 mesh)	4.57	34.6 ± 3.0[c]	1.58
	Micro-hammermill	2.00 (9 mesh)	4.66	34.8 ± 1.5[c]	1.62
		4.00 (5 mesh)	2.34	34.9 ± 1.3[c]	0.82

[1]Means with the same superscripts are not significantly different at $p \leq 0.05$.

Extraction with Alternative Solvents

The corn germ contains 80% of the total lipids in the kernel. If only the corn germ was used to extract the lipids and 90% oil recovery efficiency from germ were assumed, then approximately 72% of the total lipids can be extracted by the current technology used in industry (i.e., 80 x 0.90 = 72%). In utilizing the entire corn kernel for extraction in this study, more lipids have the potential to be recovered by the solvent since the

remaining 20% in the endosperm was also extractable. The aqueous concentrations used were the azeotropic mixtures of the solvents which are economical than their anhydrous forms. Ethanol was evaluated at 40^{0}C (Table 3) because at this temperature, the alcohol has sufficient solubility to extract all of the oil (ca 10%) while sufficient solubilities can be achieved by the other solvents even at room temperature.

Oil recoveries were calculated on the bases of both actual oil yield and residual oil content for mass balance purposes and to verify the accuracy of the data. While the trends were similar (Table 3), the oil recoveries based on residual oil content were regarded to be more reliable because the same method of crude fat analysis was performed on the same corn sample after the treatment was applied. Statistical analyses which support this contention are presented in Appendix Tables A-1, A-2, and A-3. Oil recoveries based on yield were less than those based on residual oil in almost all of the solvents. This difference may have been due to retention of some of the oil in the residues which were extracted or to losses incurred in transferring the hexane washings to another flask. Anhydrous acetone appeared to have extracted materials other than oil which contributed significantly to the oil yield. The consequences of this contamination are still unknown.

All solvents gave oil recoveries which were either nearly equal to or better than those of industry and petroleum ether (Table 3 and Figure 3). The oil extracted by the various solvents had the reddish-orange color typical of crude corn oil, except in the case of acetone and butanol:acetone:ethanol which had the clear light yellow color of refined oil. Oil extracted with aqueous butanol at its boiling point was dark. Solvents showed good oil recoveries especially at higher concentrations and temperatures. Aqueous acetone at 25^{0}C exhibited the poorest extraction among the solvents.

Statistical analysis of the main effects revealed that the kind of solvent, concentration and temperature significantly affected oil recoveries. Concentration exerted the greatest influence on the extraction yields. It should be noted from Table 3 that the

Table 3. Oil recovery from flaked corn using solvents which can be produced by cornstarch fermentation

Treatment		Mean oil yield (g/100 g dry corn)	Oil recovery[1] (%)	Mean residual oil (g/100 g dry corn)	Oil recovery[2] (%)
Solvent	Extraction temperature, °C				
Control[3]	4	4.88	100.0		100.0
Petroleum Ether	60	4.36	89.3 ± 2.3	0.35	92.8 ± 0.9
91% Isopropanol	25	3.12	64.0 ± 0.7	1.02	79.0 ± 1.2
	75	3.66	74.9 ± 1.0	0.29	94.1 ± 0.2
100% Isopropanol	25	3.50	71.7 ± 0.0	0.90	81.5 ± 0.3
	75	3.73	76.4 ± 7.8	0.21	95.6 ± 0.0
95% Ethanol	40	3.22	65.9 ± 1.3	0.79	83.8 ± 0.8
	75	3.20	65.6 ± 3.2	0.39	92.0 ± 0.5
100% Ethanol	40	3.88	79.6 ± 1.3	0.49	90.0 ± 0.3
	75	4.22	86.6 ± 1.0	0.12	97.5 ± 0.5
67% Butanol	25	3.43	70.3 ± 0.9	0.35	92.8 ± 1.5
	75	3.91	80.1 ± 3.2	0.29	94.0 ± 1.9
100% Butanol	25	3.45	70.7 ± 4.1	0.83	83.1 ± 0.3
	75	4.29	87.9 ± 0.9	0.22	95.4 ± 1.8
Butanol:Acetone:	25	4.20	86.1 ± 1.7	0.50	89.8 ± 0.1
Ethanol (6:3:1)	50	4.76	97.6 ± 0.1	0.27	94.4 ± 0.4
85% Acetone	25	3.40	69.6 ± 1.6	1.64	66.5 ± 3.2
	50	3.54	72.5 ± 2.3	0.65	86.6 ± 2.2
100% Acetone	25	6.31	129.3 ± 26.7	0.60	87.8 ± 0.1
	50	9.41	192.7 ± 26.2	0.58	88.1 ± 3.8
LSD at $p \leq 0.05$			18.19 (5.46)[4]		3.06

[1]Based on actual oil yield.

[2]Based on residual oil content.

[3]Control denotes oil recovery by Goldfisch extraction.

[4]The number in parentheses is the LSD when anhydrous acetone was excluded.

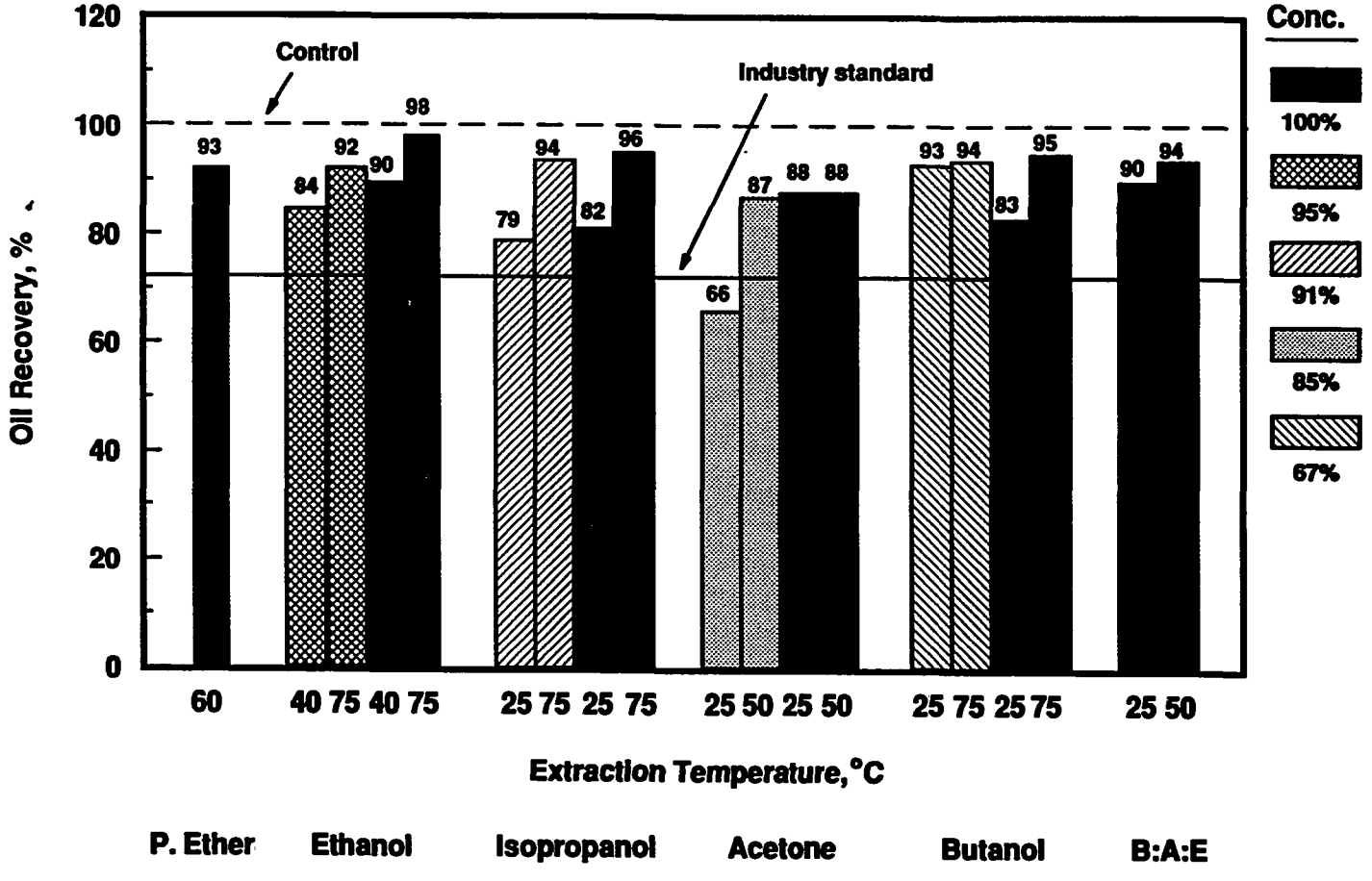


Figure 3. Comparison of solvent oil recoveries against industry practice (industry standard) and petroleum ether (control)

anhydrous solvents extracted more oil than their aqueous counterparts. As the water content increased, so did the polarities of these solvents thereby causing a corresponding decrease in oil solubilities (Harris and Hayward, 1950). Although greater oil recoveries were obtained at the higher temperatures, substantial yields of crude oil (over industry's estimated recovery of 72%) were still achieved even at ambient conditions. This finding indicates that low temperature extraction is feasible, particularly when using butanol:acetone:ethanol, ethanol, and isopropanol. The extraction capacity of each solvent varies with the nature of the solvent. Concentration and temperature provide the strongest interaction effects with the solvent.

Effect of Oil Extraction on Total Protein Content

Substantial losses in total crude protein content were observed under some conditions in corn extracted with aqueous butanol, aqueous isopropanol and aqueous ethanol (Table 4). The polarity of these alcohols were apparently favorable for co-extraction of some protein fractions with the oil. Prolamins were probably the predominant corn proteins co-extracted with the oil due to their solubility in alcohols. These proteins are hydrophobic due to the lack of charged essential amino acids. Butanol is the least polar among the three alcohols, a property which favors hydrophobic interaction with prolamins. This may explain why corn extracted with 67% butanol at 75^{0}C gave the greatest co-extraction of protein.

Higher oil extraction temperatures generally increased protein loss, particularly when the solvents were aqueous butanol, aqueous isopropanol and aqueous ethanol. Solvent concentration was a factor in protein loss when the solvents involved were butanol and isopropanol.

Protein loss was calculated on the bases of the difference between protein contents prior to and after oil recovery and of the protein content of the residue extracted with the oil. This was done to verify the accuracy of the results through the mass balance on

Table 4. Residual protein in flaked corn after oil extraction and amount of protein extracted with the oil

Treatment		Mean crude protein after oil extraction[1] (% db, ffb)	Protein loss[2] %	Mean protein extracted with oil (% db, ffb)	Protein loss[3] %
95% Ethanol	40°C	9.10 ± 0.42^{def}	7.89	0.53	5.36
	75°C	8.81 ± 0.32^{ef}	10.83	2.13	21.55
100% Ethanol	40°C	9.55 ± 0.51^{abcd}	3.34	0.05	0.51
	75°C	9.21 ± 0.21^{cdef}	6.78	0.46	4.66
91% Isopropanol (IPA)	25°C	9.78 ± 0.12^{abc}	1.01	0.26	2.63
	75°C	8.07 ± 0.04^{g}	18.32	2.46	24.90
100% IPA	25°C	9.96 ± 0.04^{a}	None	0.02	0.20
	75°C	9.84 ± 0.01^{abc}	0.40	0.18	1.82
85% Acetone	25°C	9.61 ± 0.06^{abcd}	2.73	0.33	3.34
	50°C	9.50 ± 0.07^{abcd}	3.85	1.09	11.03
100% Acetone	25°C	9.84 ± 0.01^{abc}	0.40	0.02	0.20
	50°C	9.78 ± 0.14^{abc}	1.01	0.02	0.20
67% Butanol	25°C	8.64 ± 0.45^{fg}	12.55	1.31	13.26
	75°C	7.32 ± 0.01^{h}	25.91	2.90	29.35
100% Butanol	25°C	9.78 ± 0.06^{abc}	1.01	0.01	0.10
	75°C	9.25 ± 0.07^{bcdef}	6.38	0.25	2.53
Butanol:acetone: ethanol (6:3:1)	25°C	9.49 ± 0.00^{abcde}	3.95	0.04	0.40
	50°C	9.72 ± 0.04^{abcd}	1.62	0.10	1.01
Pet. Ether	60°C	9.72 ± 0.00^{abcd}	1.62	No residue extracted	

[1]Means with the same superscript are not significantly different at $p \leq 0.05$. The symbol db denotes dry basis and ffb, fat-free basis.

[2]Based on the difference in protein content of flaked corn before and after oil extraction. Initial crude protein content was 9.88% (db, ffb).

[3]Based on % crude protein of residues extracted with the oil.

total protein content. Similar trends were observed between the two values for protein loss as influenced by the oil extraction conditions. The amount of protein lost as determined by difference was calculated by dividing the difference between the protein content of corn before and after oil extraction by the starting crude protein content. The result was a more reliable point of reference since the crude protein analysis was performed on the same corn sample and the calculations for protein loss were more direct since the difference in protein contents already represented protein loss. On the other hand, the amount of protein extracted with the oil was derived by first multiplying the weight of the solid residue by its crude protein content and then dividing the product by the weight of the flaked corn used for extraction. The result was then divided by the initial crude protein content to determine the value for protein loss. Because more calculations involved, the risk for errors is greater, thus these values could not be used with confidence for comparison of results.

Varietal Effects on Oil Extraction

The ethanol concentration selected for oil extraction was 97.5%, the mean of the aqueous azeotropic and anhydrous forms of the alcohol. Oil recovery using this solvent was expected to be nearly as good as that of the anhydrous ethanol. All three varieties had oil recoveries which were significantly greater than the 72% recovery of industry and only slightly less than the 97.5% recovery of anhydrous ethanol at 75^{0}C (Table 3) using medium-hard dent corn (Pioneer 3732). No significant difference was detected among oil yields from the three types of corn (Table 5).

Table 5. Oil and protein extracted from three corn varieties using 97.5% ethanol

Variety	Oil[1] recovery (% db)	Protein[2] recovery (% db)
Pioneer 3732	92.06 ± 1.94[a]	1.64
Pioneer 3377	96.58 ± 1.15[a]	2.32
High-Lysine	95.54 ± 0.14[a]	1.04

[1]Means with the same superscripts are not significantly different at $p \leq 0.05$.

[2]Percent protein in corn extracted with the oil.

SUMMARY AND CONCLUSIONS

Flaked corn exhibited better extraction characteristics than ground corn. Careful handling of the flakes was needed to prevent generating fines.

All solvents tested extracted oil in quantities comparable to the 72% recovered by current technology employed by industry. Acetone removed other non-oil materials which were not identified. Anhydrous solvents and high extraction temperatures recovered more oil. Low temperature extraction appears feasible when using ethanol (40^{0}C), isopropanol (25^{0}C) and butanol:acetone:ethanol (25^{0}C). Best oil colors were achieved using acetone and butanol:acetone:ethanol.

Substantial reductions in total crude protein content were observed when extracting corn with butanol, isopropanol, and ethanol, particularly when aqueous concentrations and high temperatures were used for extraction. Oil extraction using aqueous butanol at 75^{0}C produced the greatest co-extraction of crude protein.

Oil recoveries from medium-hard dent corn (Pioneer 3732), soft dent corn (Pioneer 3377) and high-lysine corn were not significantly different.

REFERENCES

AACC. 1983. Approved methods. 8th ed. American Association of Cereal Chemists, Inc., St. Paul, MN.

Beckel, A. C., P. A. Belter, and A. K. Smith. 1948a. Solvent effects on the products of soybean oil extraction. J. Am. Oil Chem. Soc. 25:7-9.

Beckel, A. C., P. A. Belter, and A. K. Smith. 1948b. The non-distillation alcohol extraction process for soybean oil. J. Am. Oil Chem. Soc. 25:10-11.

Gardner, H. K., Jr., S. P. Koltun, and H. L. E. Vix. 1968. Solvent extraction of aflatoxins from oilseeds meals. J. Agric. Food Chem. 16:990.

Gastrock, E. A., E. L. D'Aquin, E. J. Keating, V. Krishnamoorthi, and H. L. E. Vix. 1965. A mixed solvent-extraction process for cottonseed. Cereal Sci. Today 10:572.

Harris, W. D., and J. W. Hayward. 1950. Isopropanol as a solvent for extraction of cottonseed oil. III. The use of recycling to effect solvent economy. J. Am. Oil Chem. Soc. 27:273-275.

Harris, W. D., F. F. Bishop, C. M. Lyman, and R. Helpert. 1947. Isopropanol as a solvent for extraction of cottonseed oil. I. Preliminary investigations. J. Am. Oil Chem. Soc. 24:370-375.

Harris, W. D., J. W. Hayward, and R. A. Lamb. 1949. Isopropanol as a solvent for extraction of cottonseed oil. II. Separation of purified oil from the miscella. J. Am. Oil Chem. Soc. 26:719-723.

Hassanen, N. Z., L. A. Johnson, J. T. Farnsworth, and E. W. Lusas. 1985. Sequential extraction process for extracting oil and aflatoxin from cottonseed. Abstract no. 81. J. Am. Oil Chem. Soc. 62(4):639.

Haumann, B. F. 1985. Corn oil. J. Am. Oil Chem. Soc. 62(11):1524-1531.

Hron, R. J., Sr., and M. S. Kuk. 1989. Acetone extracted cottonseed meals without catty odors. J. Food Sci. 54(4):1088-1089.

Hron, R. J., Sr., S. P. Koltun, and A. V. Graci, Jr. 1982. Biorenewable solvents for vegetable oil extraction. J. Am. Oil Chem. Soc. 59(9): 674A-684A.

Johnson, L. A., and E. W. Lusas. 1983. Comparison of alternative solvents for oils extraction. J. Am. Oil Chem. Soc. 60(2):229-241.

Karnofsky, G. B. 1981. Ethanol and isopropanol as solvents for full-fat cottonseed extraction. Oil Mill Gaz. 85:34.

Norris, F. A. 1982. Extraction of fats and oils. Pages 215-244 in D. Swern, ed. Bailey's industrial oil and fat products. Vol. 2. 4th ed. John Wiley and Sons, New York.

Rao, R. K., and L. K. Arnold. 1956a. Alcoholic extraction of vegetable oils. II. Solubilities of corn, linseed and tung oils in aqueous ethanol. J. Am. Oil Chem. Soc. 33:82-84.

Rao, R. K., and L. K. Arnold. 1956b. Alcoholic extraction of vegetable oils. III. Solubilities of babassu, coconut, olive, palm, rapeseed, and sunflower seed oils in aqueous ethanol. J. Am. Oil Chem. Soc. 33:389-391.

Rao, R. K., and L. K. Arnold. 1957. Alcoholic extraction of vegetable oils. IV. Solubilities of vegetable oils in aqueous 2-propanol. J. Am. Oil Chem. Soc. 34:401-404.

Rao, R. K., and L. K. Arnold. 1958. Alcoholic extraction of vegetable oils. V. Pilot plant extraction of cottonseed by aqueous ethanol. J. Am. Oil Chem. Soc. 35:277-281.

Rao, R. K., M. G. Krishna, S. H. Zaheer, and L. K. Arnold. 1955. Alcoholic extraction of vegetable oils. I. Solubilities of cottonseed, peanut, sesame and soybean oils in aqueous ethanol. J. Am. Oil Chem. Soc. 32:420-423.

Reiners, R. A., and C. M. Gooding. 1970. Corn oil. Pages 241-262 in G. E. Inglett, ed. Corn: Culture, processing, products. Avi Publ. Co., Inc., Westport, CT.

SAS. 1987. SAS/STAT guide for personal computers. Version 6 ed. SAS Inst. Inc., Cary, NC.

Swallen, L. C. 1941. Zein - a new industrial protein. Ind. Eng. Chem. 33(3):394-398.

Vaccarino, G., and S. Vaccarino. 1961. A new industrial process for cottonseed. J. Am. Oil Chem. Soc. 38:143-147.

Watson, S. A. 1987. Structure and composition. Pages 53-82 in S. A. Watson and P. E. Ramstad, eds. Corn: Chemistry and technology. Am. Assoc. of Cereal Chem., St. Paul, MN.

Weber, E. J. 1978. The lipids of corn germ and endosperm. J. Am. Oil Chem. Soc. 56:637-641.

Youn, K. C., and D. J. Wilpers. 1981. Process for oilseed extraction with an isopropanol-based solvent. U.S. Patent No. 4,298,540.

Youngs, G. G., and H. R. Sallans. 1955. Acetone as a selective solvent for vegetable oils. J. Am. Oil Chem. Soc. 32:397-400.

PART II. THE EFFECT OF OIL EXTRACTION ON THE SOLUBILITY OF CORN PROTEINS

ABSTRACT

Protein denaturation as a consequence of oil extraction from whole corn was evaluated by determining the changes in the solubility profile of the major corn proteins. The ethanol-soluble proteins (prolamins) displayed the greatest reduction in their solubility/extractability, followed by the salt-soluble globulins. High-temperature oil extraction was more detrimental to protein solubility, especially in the case of the prolamins. Among the solvents used for oil extraction, isopropanol and ethanol have the best potential for the sequential extraction processing since they can remove comparable amounts of corn oil without significantly denaturing corn proteins.

INTRODUCTION

Although corn has relatively low protein content (9.5%, db), the volumes consumed as livestock feed and human food make it an important source of protein (Wilson, 1987; Wright, 1987). Osborne (1897) first classified corn proteins according to their solubilities in various solvents. Osborne and Mendel (1914) designated these proteins as albumins (water-soluble), globulins (soluble in dilute salt solutions), prolamins (soluble in 60-90% alcohol), and glutelins (soluble in dilute alkali or acid). Landry and Moureaux (1970) improved the extractability of the glutelins by using the reducing agent 2-mercapto-ethanol.

There is a great difference in the distribution of the types of proteins in the endosperm and the germ of corn. Endosperm proteins are mostly prolamins (particularly zein) and glutelins. Zein contains high levels of leucine, alanine, proline, phenylalanine, and glutamine but lacks the essential amino acids tryptophan and lysine and contains low amounts of threonine, valine, and the sulfur amino acids. Zein is considered to be of poor biological value (Osborne and Mendel, 1914) and the quality of endosperm proteins as a whole is inferior to that of the germ proteins. The higher nutritional value of the germ protein can be related to a better balance of essential amino acids (lysine, arginine, histidine, and aspartic acid) in the globulins and albumins, the major protein fractions in the germ (Wilson, 1987).

Corn protein fractionation is affected by temperature, presence of proteolytic enzymes (Wilson, 1987), the presence of phytate/phytic acid (Craine and Fahrenholtz, 1958; O'Dell and De Boland, 1976), and the presence or absence of salts (Nagy et al., 1941). In addition, it has been suggested that solvents for lipid extraction may affect the solubilities of the albumins and globulins so that they are extracted with the insoluble or glutelin fractions (Byers et al., 1983). Landry and Moureaux (1981) believed that lipids react with corn proteins and affect their solubilities and extractabilities.

The proposed Sequential Extraction Processing involves extraction and recovery of the proteins after oil removal. It is therefore important to determine how the oil extraction conditions affect the subsequent extractability of corn proteins in the latter steps.

Research Objectives

This study was conducted to evaluate protein loss and denaturation as a consequence of the oil extraction process. The specific objectives of the study were to identify the protein fractions which were sensitive to the oil extraction conditions, and to identify the solvent(s) which can extract the oil without significantly denaturing the proteins of corn.

MATERIALS AND METHODS

Preparation of Flaked Corn for Protein Fractionation

Flaked Pioneer 3732 corn samples defatted with ethanol, isopropanol, acetone, butanol, or butanol:acetone:ethanol (6:3:1) were desolventized and then ground using the Glenmills microhammermill IV (Glenmills Inc., Maywood, NJ). The dried ground corn samples were analyzed for moisture and crude protein contents using AACC standard procedures 44-15A and 46-13, respectively (AACC, 1983). Fifty-gram portions were taken from each treatment for removal of residual oil which was accomplished by defatting twice with petroleum ether at 4^{0}C during a 24 hr period. Continuous stirring and a solvent-to-corn ratio of 15 ml:1 g were employed. The petroleum ether was then decanted, an aliquot was taken, introduced into a tared container and then evaporated using a steam bath. The container was then dried in an oven at 100^{0}C for 30 min, cooled in a desiccator and then weighed for the amount of residual oil. The excess solvent was removed from the ground sample first by air-drying and then by vacuum-drying at 40^{0}C. This fat-free, moisture-free sample was then used as samples for protein fractionation. Unextracted ground corn was also prepared in the same manner to serve as the control. Two samples of defatted corn were used in each step of the fractionation procedure.

Protein Fractionation

The protein fractions were extracted by using the methods of Landry and Moureaux (1970) and Hu and Esen (1981). The procedure is outlined in Figure 1. The crude protein contents (N x 6.25) of the sample before fractionation, the supernatant after extraction and centrifugation, and the residue retained after centrifugation were determined by AACC standard method 46-13 (AACC, 1983). The extent of denaturation was estimated on the basis of the changes in the solubility of the major protein fractions.

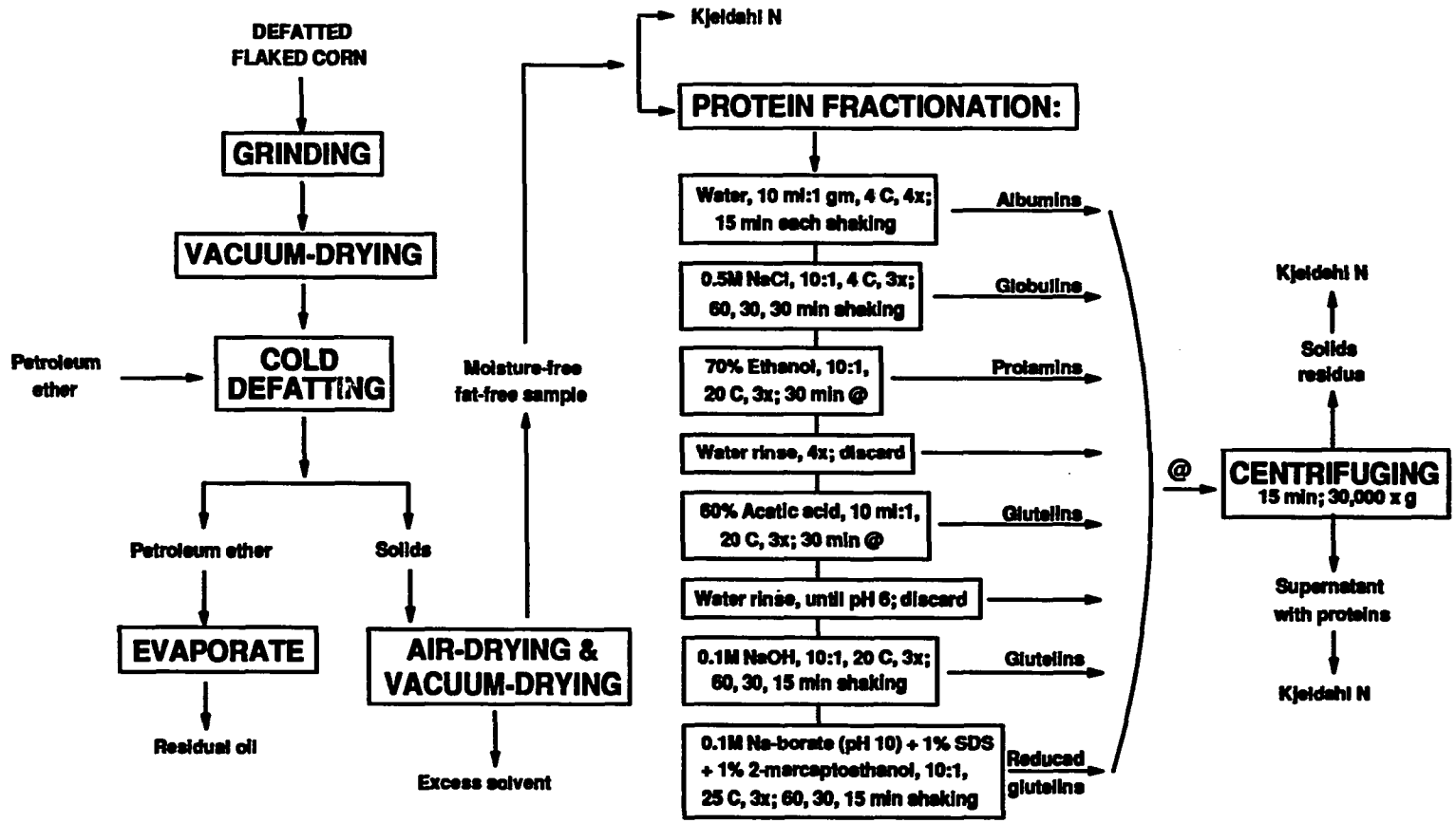


Figure 1. Procedure for sample preparation and fractionation of corn protein

Statistical Analysis

Data were analyzed using a Statistical Analysis System (SAS, 1987) program. Significant treatment effects were determined by the Analysis of Variance (ANOVA) procedure. Significant differences among treatment means within a protein class were identified using the test for Least Significant Difference (LSD). Probability levels of $p \leq 0.05$ were considered significant.

RESULTS AND DISCUSSION

Effect of Low Temperature Oil Extraction on Solubilities of Corn Protein Fractions

Acetone, butanol, and the butanol:acetone:ethanol mixture caused significant reductions in the amount of extractable proteins from nearly all the fractions (Table 1). Only the glutelins, the alkali-soluble proteins, appeared to be stable against the conditions employed. The high F-values for the salt-soluble (globulins) and ethanol-soluble (prolamins) proteins indicated that these fractions were sensitive to the solvent even when low temperatures (25-40^{0}C) were employed for oil extraction. Aqueous butanol had the most deleterious effect on the protein fractions, particularly on the albumins, globulins and prolamins. Byers et al. (1983) reported that using butanol as a defatting solvent prior to protein extraction rendered albumins and globulins in wheat unextractable and caused an increase in N content in the residue. No such increase was observed in these residues or in the other fractions to indicate denaturation or cross-contamination (Wilson, 1987). Decreasing amounts of the reduced proteins (with 2-mercaptoethanol) also indicate an increasing degree of denaturation (Hu and Esen, 1981), in which case 91% isopropanol and the butanol:acetone:ethanol mixture were the most damaging to the proteins. However, in this study, there was no corresponding increase in the residue proteins to confirm this. It is probable that the reduction in the amounts of zein occurred because of co-extraction with the oil since the alcohols, acetone and their mixture are all capable of extracting the proteins (Byers et al., 1983; Swallen, 1941); thus, there was less protein available for the fractionation studies.

Effect of High Temperature Oil Extraction on Solubilities of Corn Protein Fractions

Only the acid-soluble proteins were not affected by the solvent treatments when extracting oil from whole corn at high temperatures (Table 2). The F-values obtained for the other fractions were higher than those given in Table 1, indicating that high-

Table 1. Protein profiles after oil extraction of flaked corn at low temperatures (25-40°C)

Oil extraction solvent	Temp. °C	Mean crude protein retained in the fractions[1] (% of total available protein)						
		A	B	C	D	E	F	G
Control[2]	4	11.13	9.56	16.70	13.36	24.64	11.04	15.89
95% Ethanol	40	10.28	11.00	16.76	11.12	25.18	9.78	14.42
100% Ethanol	40	10.95	8.51	12.35	7.46	26.10	8.16	13.50
91% IPA	25	9.78	10.53	20.04	10.42	27.10	5.80	12.32
100% IPA	25	10.30	8.28	19.02	12.16	25.01	9.79	11.86
85% Acetone	25	8.59	9.47	16.91	14.56	26.17	8.14	11.95
100% Acetone	25	8.08	5.24	14.84	10.16	21.16	8.41	12.11
67% Butanol	25	5.66	5.18	13.08	10.44	23.81	9.36	14.64
100% Butanol	25	8.16	6.70	15.28	10.23	22.74	8.58	10.32
B:A:E[3]	25	8.28	6.38	13.80	9.91	18.64	6.29	14.16
LSD $p \leq 0.05$		2.82	2.44	3.32	2.95	6.41	2.33	2.69
F-value		3.55*	7.47**	5.60**	4.49**	1.61ns	4.65*	3.87*

[1]A denotes water-soluble fraction (albumins), B, salt-soluble (globulins), C, soluble in 70% ethanol (prolamins), D, acid-soluble (glutelins), E, soluble in 0.1 M NaOH (glutelins), F, soluble in 0.1 M Na-borate + 1% SDS + 1% 2-mercaptoethanol (reduced glutelins), and G, residue after fractionation.

[2]Petroleum ether (cold defatting).

[3]Butanol:acetone:ethanol.

*Significant at $p \leq 0.05$.

**Significant at $p \leq 0.01$.

nsNot significant.

temperature extraction has more detrimental effect on protein solubility/extractability. The application of heat causes structure modifications of proteins which reduce solubility, due to the exposure of hydrophobic groups and the aggregation of the unfolded protein molecules.

Zein was the most severely affected fraction. The ten-fold increase in its F-value further underscored the negative effect of high temperature on protein extractability. Zein is soluble in aqueous alcohols (Swallen, 1941) and the elevated temperature may have increased its solubility (Cheftel et al., 1985), resulting in significant quantities being co-extracted with the oil. However, denaturation may have also occurred since there were notable increases in the amount of residual proteins (fraction G) when aqueous butanol and isopropanol were the solvents (Byers et al., 1983). Concentration effects also became significant under this condition. Less protein was generally extracted from corn treated with the aqueous solvents. The detrimental effects of certain alcohols and acetone on protein solubility are attributed to their abilities to lower the dielectric constant of the medium in which the protein is dissolved. The resulting decrease in the electrostatic forces of repulsion among the protein molecules contributes to a decrease in their solubility (Cheftel et al., 1985).

Potential Solvents for Oil and Protein Extraction

Almost all tested solvents extracted oil in quantities which were better than the 72% recovery for industry (Table 3). The sole exception was aqueous acetone at 25^{0}C. More oil was extracted at the higher temperatures (50-75^{0}C) and, generally, with anhydrous solvents. Aqueous ethanol (75^{0}C), anhydrous ethanol, isopropanol (75^{0}C), butanol, and butanol:acetone:ethanol (50^{0}C) had oil recoveries which were nearly equal to or better than the recovery for petroleum ether at 60^{0}C. Still more oil, however, was obtained by cold-defatting of the corn with petroleum ether. This was probably due to the large surface area of the corn in contact with the solvent (corn was ground), the use of

Table 2. Protein profiles after oil extraction of flaked corn at high temperatures (50-75°C)

Oil extraction solvent	Temp. °C	Mean crude protein retained in the fractions[1] (% of total available protein)						
		A	B	C	D	E	F	G
Control[2]	60	8.26	5.39	13.60	10.68	28.18	9.72	16.16
95% Ethanol	75	10.86	8.66	6.06	12.88	29.07	9.60	17.68
100% Ethanol	75	11.60	7.61	9.18	12.32	28.99	7.01	15.46
91% IPA	75	9.11	6.78	6.62	12.55	33.24	11.60	19.31
100% IPA	75	10.77	5.07	17.42	12.64	23.31	8.99	11.54
85% Acetone	50	8.91	8.67	10.20	14.67	23.36	6.20	12.98
100% Acetone	50	6.84	5.42	16.42	10.89	16.46	6.65	11.81
67% Butanol	75	6.15	2.90	2.87	7.10	22.65	6.46	22.88
100% Butanol	75	9.17	6.50	14.65	11.79	21.79	6.87	9.24
B:A:E[3]	50	8.27	5.61	13.92	11.18	17.67	6.90	13.12
LSD $p \leq 0.05$		2.49	2.03	2.06	4.93	6.74	2.79	3.09
F-value		5.00**	8.97**	54.85**	1.51ns	5.46**	4.84**	15.55**

[1]A denotes water-soluble fraction (albumins), B, salt-soluble (globulins), C, soluble in 70% ethanol (zein), D, acid-soluble (glutelins), E, soluble in 0.1 M NaOH (glutelins), F, soluble in 0.1 M Na-borate + 1% SDS + 1% 2-mercaptoethanol (reduced glutelins), and G, residue after fractionation.

[2]Petroleum ether.

[3]Butanol:acetone:ethanol.

*Significant at $p \leq 0.05$.

**Significant at $p \leq 0.01$.

nsNot significant.

continuous stirring, the longer extraction period (24 hr), and the much higher 15 ml:1 g solvent-to-corn ratio. In contrast, petroleum ether recovered oil from flaked whole corn at 60^{0}C by percolation extraction for 90 min using a 2:1 (w/w) solvent-to-corn ratio.

The ability of solvents to extract oil without extracting or denaturing the proteins is an important consideration for the proposed sequential extraction processing of corn because of the desire to produce the maximum yield of corn proteins with the highest retention of their functional properties. The potential protein recovery was calculated by adding the amounts of the water-soluble, ethanol-soluble and alkali-soluble fractions obtained in the solubility experiments. These are the proteins which were expected to be recovered from defatted, flaked, undegermed corn when an aqueous mixture of alcohol and alkali was used to extract the proteins.

The expected protein recovery was markedly reduced when high temperatures were used for oil extraction by aqueous solvents (Table 3). There was no significant difference between expected protein recoveries from corn defatted with anhydrous solvents at either low or high temperature. The amounts of protein which were extracted from corn defatted with ethanol, isopropanol, or aqueous acetone (25^{0}C) were almost as much as, if not more than, the expected protein recovery from corn defatted with petroleum ether. Corn extracted with aqueous butanol at 75^{0}C had the lowest expected protein recovery.

Ethanol and isopropanol appeared to have the best potential to recover oil with minimum extraction/denaturation of protein. Aqueous acetone (25^{0}C) had a high expected protein recovery but its oil yield was very poor. Anhydrous acetone, butanol, and butanol:acetone:ethanol showed excellent oil recoveries but caused considerable reductions in the extractability of the water-soluble (albumins), alcohol-soluble (zein), and alkali-soluble (glutelins) proteins from corn.

Table 3. Summary of oil and expected protein recoveries using alternative solvents

Solvent	Temp. °C	Oil recovery (%)	Expected protein recovery[a], (%)
Control (P. Ether)	4	100.00	52.5 ± 0.2
	60	92.8 ± 0.9	50.0 ± 2.1
95% Ethanol	40	83.8 ± 0.8	52.2 ± 0.9
	75	92.0 ± 0.5	46.0 ± 0.3
100% Ethanol	40	90.0 ± 0.3	49.4 ± 2.0
	75	97.5 ± 0.5	49.8 ± 2.0
91% Isopropanol	25	79.0 ± 1.2	56.9 ± 2.8
	75	94.1 ± 0.2	49.0 ± 1.7
100% Isopropanol	25	81.5 ± 0.3	54.3 ± 0.6
	75	95.6 ± 0.0	51.5 ± 0.8
85% Acetone	25	66.5 ± 3.2	51.7 ± 5.9
	50	86.6 ± 2.2	42.5 ± 6.0
100% Acetone	25	87.8 ± 0.8	44.1 ± 3.4
	50	88.1 ± 3.8	39.7 ± 0.4
67% Butanol	25	92.8 ± 1.5	42.6 ± 0.3
	75	94.0 ± 1.9	31.6 ± 7.8
100% Butanol	25	83.1 ± 0.3	46.2 ± 6.8
	75	95.4 ± 1.8	45.6 ± 1.1
Butanol:acetone: ethanol (6:3:1)	25	89.8 ± 0.1	40.7 ± 3.4
	50	94.4 ± 0.4	39.8 ± 4.5
LSD at $p \leq 0.05$		3.06	7.35

[a]Sum of water-soluble (fraction A), ethanol-soluble (fraction C), and 0.1 M NaOH-soluble (fraction E) proteins from Tables 1 and 2.

SUMMARY AND CONCLUSIONS

Acetone, butanol, and butanol:acetone:ethanol (6:3:1) reduced the solubility profiles of the different protein classes in the corn, particularly when higher temperatures (50-75^{o}C) were employed for oil extraction. Among the classes of proteins, the extractability of the ethanol-soluble fraction (prolamin) was the most severely affected by the oil extraction treatments, followed by the salt-soluble globulins. High-temperature oil extraction was particularly detrimental to the recovery of zein. The greatest decrease in the solubilities of the proteins was observed in corn extracted with aqueous butanol at 75^{o}C.

Ethanol and isopropanol are potential solvents for the sequential extraction of oil and protein from flaked undegermed corn. Both are capable of extracting oil with minimal denaturation of the corn proteins.

REFERENCES

AACC. 1983. Approved methods. 8th ed. American Association of Cereal Chemists, Inc., St. Paul, MN.

Byers, M., B. J. Miflin, and S. J. Smith. 1983. A quantitative comparison of the extraction of protein fractions from wheat grain by different solvents, and of the polypeptide and amino acid composition of the alcohol-soluble proteins. J. Sci. Food Agric. 34:447-462.

Cheftel, J. C., J-L. Cuq, and D. Lorient. 1985. Amino acids, peptides, and proteins. Pages 245-370 in O. R. Fennema, ed. Food Chemistry. 2nd ed. Marcel Dekker, Inc., New York, NY.

Craine, E. M., and K. E. Fahrenholtz. 1958. The proteins in water extracts of corn. Cereal Chem. 35:245-259.

Hu, B., and A. Esen. 1981. Heterogeneity of soybean seed proteins: one-dimensional electrophoretic profiles of six different solubility fractions. J. Agric. Food Chem. 29:497-501.

Landry, J., and T. Moureaux. 1970. Heterogeneite des glutelines du grain de mais: extraction selective et composition en acides amines des trois fractions isolees. Bull. Soc. Chim. Biol. 52:1021-1037.

Landry, J., and T. Moureaux. 1981. Physicochemical properties of maize glutelins as influenced by their isolation conditions. J. Agric. Food Chem. 29:1205-1212.

Nagy, D., W. Weidlein, and R. M. Hixon. 1941. Factors affecting the solubility of corn proteins. Cereal Chem. 18:514-523.

O'Dell, B. L., and A. De Boland. 1976. Complexation of phytate with proteins and cations in corn germ and oilseed meals. J. Agric. Food Chem. 24:804-808.

Osborne, T. B. 1897. The amount and properties of the proteids of the maize kernel. J. Am. Chem. Soc. 19:525-532.

Osborne, T. B., and L. B. Mendel. 1914. Nutritive properties of proteins of the maize kernel. J. Biol. Chem. 18:1-16.

SAS. 1987. SAS/STAT guide for personal computers. Version 6 ed. SAS Inst., Inc., Cary, NC.

Swallen, L. C. 1941. Zein - a new industrial protein. Ind. Eng. Chem. 33(3):394-398.

Wilson, C. M. 1987. Proteins of the kernel. Pages 273-310 in S. A. Watson and P. E. Ramstad, eds. Corn: Chemistry and technology. American Association of Cereal Chemists, Inc., St. Paul, MN.

Wright, K. N. 1987. Nutritional properties and feeding values of corn and its by-products. Pages 447-451 in S. A. Watson and P. E. Ramstad, eds. Corn: Chemistry and technology. American Association of Cereal Chemists, Inc., St. Paul, MN.

PART III. EXTRACTION OF PROTEIN FROM FLAKED DEFATTED WHOLE CORN USING ALKALI/ETHANOL

ABSTRACT

Mixtures containing 0-65% (v/v) ethanol in 0.075 M, 0.100 M, and 0.125 M NaOH were evaluated for their abilities to extract protein from flaked solvent-defatted undegermed medium-hard dent, soft dent, and high-lysine corn. Maximum total protein contents for medium-hard dent and soft dent corns were obtained using either 45% or 15% ethanol with 0.100 M NaOH, while for high-lysine corn, the highest protein yields were attained using either 100% (v/v) 0.125 M NaOH or 45% ethanol with 0.125 M NaOH. The two points of maximum protein recoveries suggest the possibility of extracting two major kinds of proteins. The mixture containing 45% ethanol:55% 0.100 M NaOH was selected as the optimum solvent for protein extraction.

The effects of four temperatures (25, 45, 50, and 60^{o}C) on protein yields were also determined. Higher yields were recovered as temperature increased. No significant difference was detected between 50^{o}C or 60^{o}C.

Sonication (10KHz) and homogenization treatments were evaluated as means of improving protein extractability. Neither of these two methods significantly increased the amount of total protein extracted by the ethanol/alkali mixture. Extended treatments reduced protein recovery.

INTRODUCTION

The distribution of corn proteins varies among the parts of the kernel. The endosperm contains 75% of the total nitrogen while the germ accounts for 22% of the total nitrogen in the corn. The remainder is found in the pericarp and tipcap (Earle et al., 1946).

Landry and Moureaux (1980, 1981) fractionated the proteins of both the endosperm and the germ. They suggested two classifications for these fractions: 1) basic or metabolically essential proteins (globulins, G-3 glutelins and residue proteins) and 2) endosperm-specific proteins (zein and the G-1 and G-2 glutelins).

The predominant endosperm proteins, zein and glutelin, are storage proteins. They comprise 40% and 37%, respectively, of the grain nitrogen (Landry and Moureaux, 1970). Zein is located exclusively in subcellular structures called protein bodies (Duvick, 1961), which are tightly packed against starch granules in normal horny endosperm. The diameters and quantities of protein bodies change dramatically in genetically modified corn varieties (Wolf et al., 1969; Christianson et al., 1974). The protein bodies and the starch grains are surrounded by matrix proteins which have been associated with the glutelins (Christianson et al., 1969).

Albumins and globulins are minor components of corn endosperm protein, but they constitute 28% and 24%, respectively, of the germ protein (Paulis and Wall, 1969). They include biologically important proteins such as enzymes, membrane protein, glycoproteins and nucleoproteins. Zein is a negligible component of germ protein. Khavkin et al. (1978) suggested that the globulins were the major storage proteins of the germ.

Studies on corn proteins have focused mostly on zein and glutelin. Zein is deficient in the essential amino acids lysine and tryptophan, and, therefore, is considered to be of poor nutritional value (Osborne and Mendel, 1914). The biological value of glutelin is intermediate between the salt-soluble globulins and zein (Wall and Paulis, 1978).

Swallen (1941) summarized the properties and uses of zein, and compared the zein-extraction capabilities of several alcohols, ketones and other solvents. Paulis (1982) and Landry et al. (1983) described methods of separating glutelin sub-groups using alcohols combined with salts or reducing agents. A few researchers have evaluated various conditions for the alcohol-extraction of the endosperm proteins. Ethanol has been frequently used and the reported optimum concentration has ranged from 55-70% (Russell and Tsao, 1982; Turner et al., 1965). Russell and Tsao (1982) evaluated a process which combined elements of dry corn milling to separate fiber and germ, followed by extraction with alcohol and then alkali to remove zein and glutelins from corn endosperm. The total protein recovery was about 80%. Lusas et al. (1985) reported that extraction efficiency of endosperm proteins can be as much as 85% if the pH of the aqueous phase is adjusted to 11.5. Concon (1973) claimed 97% of the zein can be recovered if NaOH is added after pre-solubilization of the protein in 70% ethanol. Temperatures close to 25^{0}C resulted in minimal denaturation of the endosperm proteins (Chen and Houston, 1970; Concon, 1973; Fellers et al., 1966; Russell and Tsao, 1982; Turner et al., 1965). The effects of pH, solvent:solids ratio, extraction time, and stirring have also been investigated (Chen and Houston, 1970; Fellers et al., 1966; Nielsen et al., 1970; Russell and Tsao, 1982; Turner et al., 1965; Wu and Sexson, 1976).

Albumins and globulins are good dietary sources of essential amino acids (Wilson, 1987), but studies on their recoveries from corn are lacking. It is important that these fractions be included in the extraction of endosperm proteins because almost complete removal of protein is required to maximize by-product return and produce high quality starch and corn syrups. Recent studies presented possible methods of increasing protein recovery. Lawhon (1986) reported that sonication (20KHz) increased protein yields. Huster et al. (1983) and Meuser and German (1984) suggested that homogenization may be incorporated into conventional wet milling to improve the separation of protein from starch and to reduce steeping times.

Research Objectives

This study was undertaken to evaluate the feasibility of sequentially extracting oil and protein from flaked undegermed corn using ethanol. The specific objectives were to establish the optimum conditions for the extraction and recovery of corn protein, and to examine the potential for sonication and homogenization to enhance protein yields.

MATERIALS AND METHODS

Preparation of Corn for Extraction

Three corn varieties were evaluated for oil and protein extraction by simulation of the sequential extraction process. The varieties were Pioneer 3732 (medium-hard dent corn, Dept. of Ag. Engineering Grain Quality Laboratory, Iowa State University, Ames, IA), Pioneer 3377 (soft dent corn, Pioneer Hi-Bred International, Inc., Johnston, IA) and high-lysine corn (Crow's Hybrid Seed Co., Milford, IL). Triplicate subsamples of 400 gms each were taken from each variety. The undegermed corn samples were coarsely cracked and then flaked using a Roskamp rollermill (Model K, Roskamp Mfg., Waterloo, IA). The samples were dried to a moisture content of about 4% in a forced-air convection oven. Each corn replicate was transferred into a labeled plastic storage bag which was then sealed and stored in a desiccator until used.

Small portions of each corn sample were analyzed in triplicate for initial moisture content, crude free fat, and crude protein using AACC standard methods 44-15A, 30-20, and 46-08, respectively (AACC, 1983).

Determination of Optimum Solvent for Protein Removal

Oil extraction Oil from dried flaked whole corn was extracted with 97.5% ethanol at 75^{0}C using the procedure developed by Hassanen et al. (1985). The defatted corn was then air-dried and ground through an 11-mesh sieve in a Glenmills microhammermill IV (Glenmills, Inc., Maywood, NJ). After moisture, crude protein, and residual oil contents of these ground defatted corn samples were determined, the samples were stored in sealed polyethylene bags in the cold room (5^{0}C) until used. Oil was recovered from the miscella with a rotary evaporator. Further separation between oil and any solid residue was accomplished by washing with petroleum ether and then evaporating the solvent in a water bath. Oil and residue yields among the three varieties were recorded and

compared. Residual oil and crude protein contents of the defatted meal were determined by AACC standard procedures 30-20 and 46-08, respectively (AACC, 1983).

Protein extraction The levels of ethanol and NaOH solution in the mixture were variables studied for protein extraction. Seven concentrations of ethanol were used [0, 15, 25, 35, 45, 55, and 65% (v/v)] in combination with three concentrations of NaOH (0.075, 0.100, and 0.125 M). The experimental scheme is presented in Figure 1. The solvent was pre-heated to 50^{0}C in a water bath and then added to the defatted ground corn in a 250-ml centrifuge bottle using a 15 ml:1 g solvent:corn ratio. The bottles were covered tightly and then fastened securely to racks of a Fisher Versa-Bath S shaker bath maintained at 50^{0}C. The bottles were shaken for 2 hr at the rate of 130 rpm. After extraction, the bottles were wiped dry and then centrifuged for 15 min at 2200 x g and 20^{0}C in a Sorvall Superspeed RC2-B centrifuge (Ivan Sorvall Inc., Newtown, CT). The supernatant with the protein extract was decanted into a flask and a 15 ml aliquot was removed for Kjeldahl N determination by using a Tecator Kjeltec system. The protein yields, as well as the extraction efficiencies of the treatments, were calculated and compared. The amount of residual protein was determined by difference. All protein extractions and Kjeldahl N analyses were carried out in triplicate.

Determination of Optimum Extraction Temperature

The protein was extracted from defatted ground corn (< 4% moisture content) using 45% ethanol:55% 0.100 M NaOH at 25, 45, 50, and 60^{0}C. The solvent was preheated, when required, and added to the samples at a ratio of 15 ml:1 g. Extraction was carried out in triplicates for 2 hr after oil extraction. The N content of the supernatant was analyzed by the AACC standard method 46-08 (AACC, 1983), and protein recoveries were evaluated.

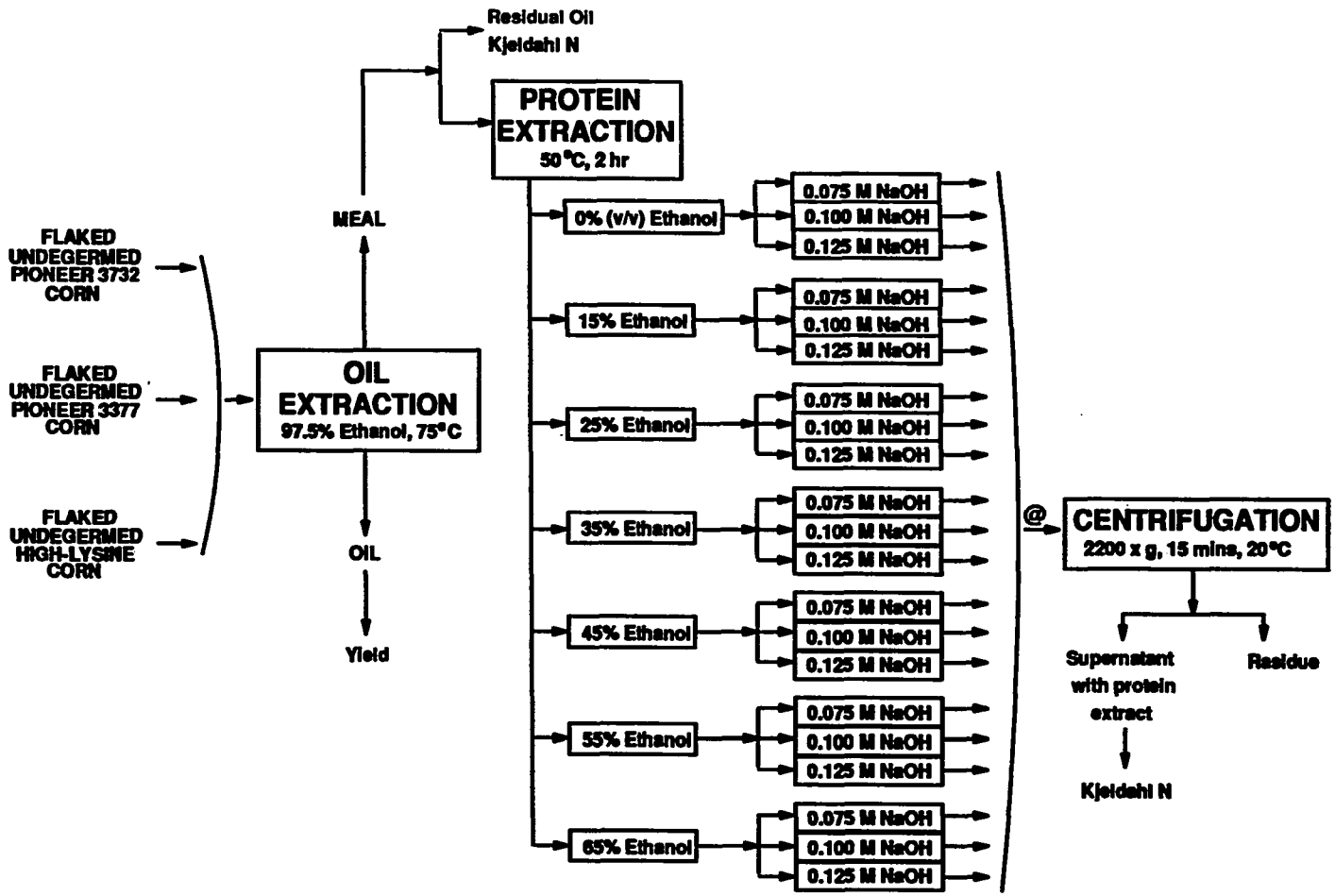


Figure 1. Procedure for evaluating ethanol:NaOH mixtures as solvents for protein extraction from flaked defatted corn

Treatment with Sonication or Homogenization

Corn preparation Pioneer 3732 corn was dried, flaked, defatted and analyzed for moisture, crude protein and crude fat contents as described in the preceding sections.

Sonication A laboratory 10KHz sonicator (Swen Sonic Corp., Sonic Energy Products, Davenport, IA) was used in these experiments. The equipment operated on 350 watts power and consisted of two magnetostrictive transducers, each having the dimensions 150 mm x 230 mm. The width of the test cell (distance between the two transducers) was 16 mm (5/8"). The extracting solvent, 45% ethanol:55% 0.100 M NaOH, was preheated to 55^{0}C and added to the defatted ground corn in the amount of 15 ml/g of corn. The mixture was then poured in the test cell of the sonicator. Sonication was conducted at 50%, 75%, and 100% power for periods ranging from 1 sec to 5 min (Figure 2). The sample was drained from the chamber into a 250-ml centrifuge bottle, capped tightly, and was extracted at 55^{0}C following the procedure described in the section on protein extraction.

Homogenization The defatted ground corn samples were first extracted with 45% ethanol:55% 0.100 M NaOH at 55^{0}C for 2 hr in a shaking water bath. The samples were subjected to two-stage homogenization at pressures of 0.70 kg/mm^{2} (1000 psi) and 3.16 kg/mm^{2} (4500 psi) using a Gaulin Model 15 M laboratory homogenizer (Gaulin Corp., Everett, MA). The homogenized corn:solvent slurries were returned to the shaker bath for an additional 15 min extraction at 55^{0}C. The slurries were then centrifuged at 2200 x g for 15 min (Figure 3).

Kjeldahl N determinations were performed on the supernatants following AACC standard method 46-08 (AACC, 1983). Crude protein contents (N x 6.25) and yields were calculated and compared.

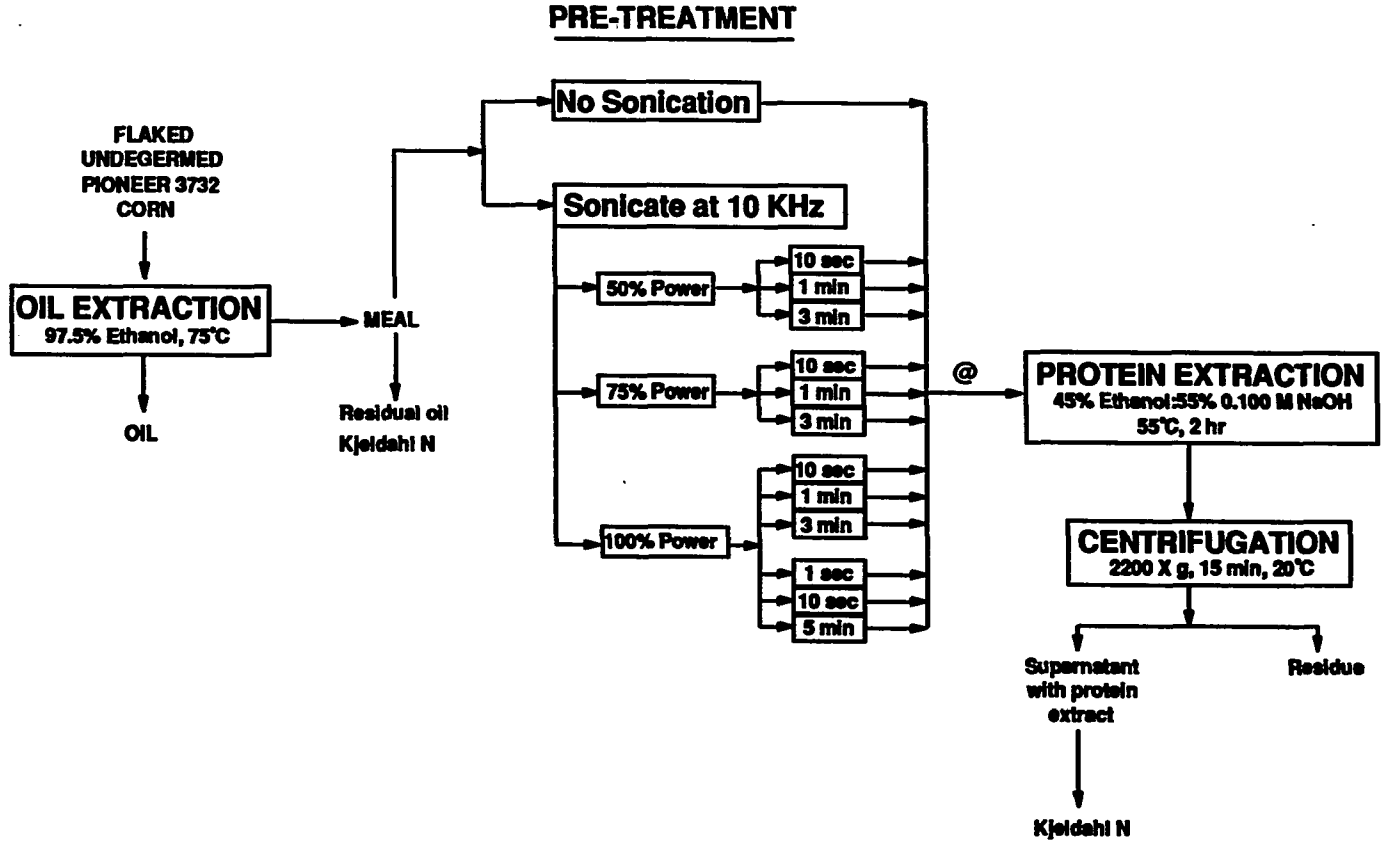


Figure 2. Experimental procedure for determining the effects of sonication on protein extraction

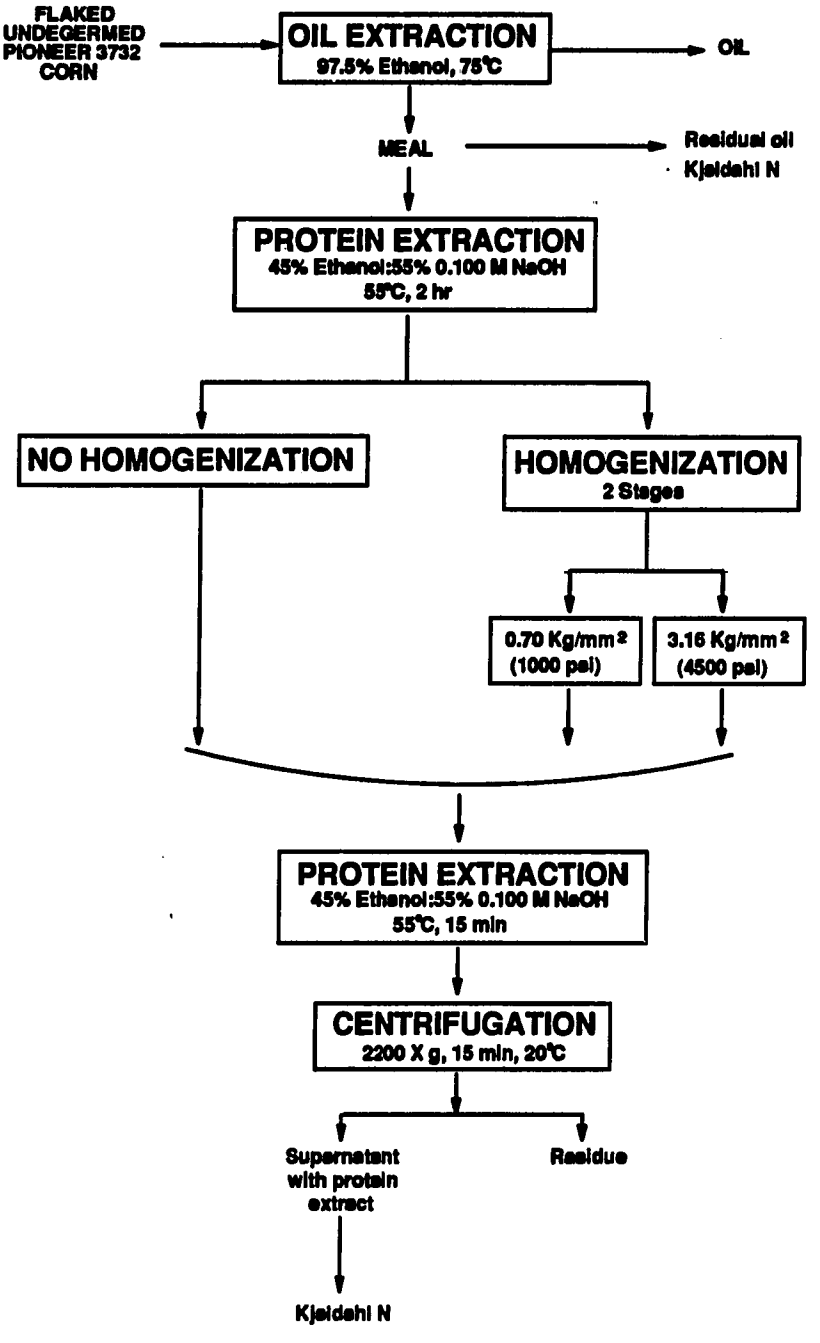


Figure 3. Experimental procedure for determining the effects of homogenization on protein extraction

Statistical Analyses

The data were analyzed using a Statistical Analysis Systems program (SAS, 1987). Significant differences were distinguished using Duncan's Multiple Range Test or the Least Significant Difference (LSD). Other main and interaction effects were detected by the Analysis of Variance (ANOVA) procedure. Probability levels of $p \leq 0.05$ were deemed significant.

RESULTS AND DISCUSSION

Oil Extraction

There were notable changes in the moisture, crude fat, and crude protein contents of medium-hard dent corn (Pioneer 3732), soft dent corn (Pioneer 3377) and high-lysine corn (Table 1). The increase in moisture/volatile content may be the result of absorption of moisture from the solvent. However, it is more likely that the rise in moisture content as determined by the oven method is due to the incomplete evaporation of ethanol during air-drying. The small amount of residual oil in the defatted meal indicated excellent oil extraction efficiency for the 97.5% ethanol. The crude oil recoveries were 94%, 97% and 96% from Pioneer 3732, Pioneer 3377 and high-lysine corn, respectively. The reduction in crude protein content in the defatted meal has been attributed to co-extraction of some proteins with the oil due to their solubility in ethanol.

Table 1. Proximate analysis of flaked undegermed corn varieties before and after extraction of oil with 97.5% ethanol at 75°C

Variety	Volatile content[a] (%)		Crude fat (% db)		Crude protein (% db)	
	Before	After	Before	After	Before	After
Pioneer 3732	2.53	7.11	4.10	0.27	9.58	8.83
Pioneer 3377	4.18	7.90	4.08	0.14	9.44	8.70
High-lysine corn	3.90	6.28	4.04	0.18	9.20	8.79

[a]Mean of 3 sample determinations.

Selection of Optimum Solvent

The protein yields and extraction efficiencies for different pretreatments are shown in Table 2. The results of the statistical analyses performed on the extraction efficiencies of the various treatments are reported in Appendix Tables A-4 and A-5. Corn variety, the concentration of ethanol in the mixture, and the concentration of NaOH strongly influenced the amount of protein extracted. The interaction effects among these factors were also significant.

Significantly higher crude protein yields were obtained from medium-hard dent corn (Pioneer 3732) and high-lysine corn than from soft dent corn (Pioneer 3377). Total protein content has been shown to be linearly related to the amount of horny endosperm in the kernel (Hamilton et al., 1951; Hinton, 1953). Medium-hard dent corn contains much higher proportion of horny endosperm compared to the other two types. This may explain the protein yield difference between hard dent and soft dent corn. Similar results were expected between high-lysine and soft dent corn in terms of total protein yields. The higher protein recovery from high-lysine corn may be due to other nitrogenous components available for extraction aside from the proteins which comprise the horny endosperm.

The ethanol concentration of the mixture with NaOH showed the greatest effect on protein recovery (Figures 4, 5, and 6). The highest protein yields were obtained with 45% (v/v) ethanol. Fifteen percent ethanol also extracted substantial quantities of crude protein from Pioneer 3732 (medium-hard dent corn) and Pioneer 3377 (soft dent corn). For high-lysine corn, the second highest extraction efficiency resulted from the use of just aqueous NaOH. Increasing the concentration of NaOH from 0.075 M to 0.100 M significantly increased the protein yield. No enhancement of protein extraction was gained by using 0.125 M NaOH. All three varieties exhibited two sets of conditions for maximum protein recovery. These twin conditions suggest the probability of extracting

Table 2. Protein yields and extraction efficiencies of three corn varieties extracted with ethanol:NaOH mixtures

Solvent			Pioneer 3732		Pioneer 3377		High-lysine	
Ethanol (% v/v)	NaOH (% v/v)	(Conc.)	Protein yield[a] (% db, ffb)[c]	Protein recovery[b] (%)	Protein yield (% db, ffb)	Protein recovery (%)	Protein yield (% db, ffb)	Protein recovery (%)
0	100	(0.075 M)	5.14 ± 0.28	58.2 ± 1.6	5.42 ± 0.19	57.8 ± 2.0	6.52 ± 0.24	69.7 ± 1.2
0	100	(0.100 M)	4.89 ± 0.16	55.1 ± 3.0	5.48 ± 0.45	58.4 ± 4.8	7.18 ± 0.33	74.7 ± 3.9
0	100	(0.125 M)	5.45 ± 0.57	61.6 ± 1.7	4.42 ± 0.25	47.1 ± 2.7	6.96 ± 0.19	75.1 ± 2.6
15	85	(0.075 M)	5.11 ± 0.24	63.1 ± 1.4	6.52 ± 0.33	69.4 ± 3.6	5.17 ± 0.28	55.5 ± 5.6
15	85	(0.100 M)	5.64 ± 0.32	69.7 ± 3.4	6.58 ± 0.34	70.1 ± 3.8	5.92 ± 0.34	63.5 ± 6.5
15	85	(0.125 M)	5.67 ± 0.27	70.1 ± 3.3	6.90 ± 0.19	73.4 ± 1.9	6.61 ± 0.64	70.9 ± 9.2
25	75	(0.075 M)	3.81 ± 0.17	47.1 ± 3.5	4.98 ± 0.32	53.1 ± 3.4	5.74 ± 0.13	61.4 ± 3.9
25	75	(0.100 M)	4.01 ± 0.07	49.6 ± 2.2	5.47 ± 0.26	58.2 ± 2.8	5.64 ± 0.16	60.3 ± 1.4
25	75	(0.125 M)	4.18 ± 0.18	51.7 ± 2.9	6.06 ± 0.13	64.6 ± 1.5	5.81 ± 0.13	62.2 ± 1.7
35	65	(0.075 M)	5.34 ± 0.06	66.1 ± 2.7	4.48 ± 0.22	47.8 ± 2.4	5.86 ± 0.07	62.7 ± 2.7
35	65	(0.100 M)	6.05 ± 0.32	74.7 ± 2.7	4.66 ± 0.16	49.6 ± 1.8	6.02 ± 0.41	64.3 ± 3.8
35	65	(0.125 M)	4.03 ± 0.15	49.8 ± 3.6	4.51 ± 0.30	48.1 ± 3.1	3.70 ± 0.14	39.6 ± 3.1
45	55	(0.075 M)	5.22 ± 0.30	64.5 ± 1.3	6.30 ± 0.29	67.1 ± 3.0	6.21 ± 0.24	66.4 ± 2.2
45	55	(0.100 M)	5.82 ± 0.22	71.9 ± 2.1	6.68 ± 0.10	71.2 ± 1.1	6.55 ± 0.22	70.1 ± 3.1
45	55	(0.125 M)	5.72 ± 0.11	70.7 ± 1.6	6.77 ± 0.05	72.1 ± 0.4	7.00 ± 0.08	75.0 ± 3.6
55	45	(0.075 M)	4.42 ± 0.09	54.7 ± 2.1	3.58 ± 0.08	38.2 ± 0.9	4.84 ± 0.00	51.8 ± 2.6
55	45	(0.100 M)	5.02 ± 0.13	62.1 ± 2.4	3.69 ± 0.09	39.2 ± 1.0	5.14 ± 0.31	55.0 ± 3.4
55	45	(0.125 M)	4.91 ± 0.25	60.7 ± 5.1	4.18 ± 0.15	44.6 ± 1.7	5.44 ± 0.26	58.3 ± 5.6

65	35	(0.075 M)	2.95 ± 0.41	36.4 ± 6.0	3.50 ± 0.09	37.3 ± 0.9	2.27 ± 0.49	24.5 ± 6.2
65	35	(0.100 M)	3.08 ± 0.04	38.1 ± 1.8	3.87 ± 0.26	41.2 ± 3.8	2.24 ± 0.36	24.0 ± 3.9
65	35	(0.125 M)	3.87 ± 0.10	47.8 ± 2.5	3.93 ± 0.26	41.8 ± 2.8	3.89 ± 0.07	41.6 ± 1.9
LSD at $p \leq 0.05$			0.41	4.86	0.39	4.22	0.47	6.90
F-value			38.99**	40.79**	72.86**	71.17**	72.73**	38.08**

[a]Mean of 3 determinations.

[b]Based on initial crude protein contents of 8.83% (Pioneer 3732), 8.70% (Pioneer 3377) and 8.79% (High-lysine), db, ffb.

[c]Db denotes dry basis; ffb denotes fat-free basis.

**Significant at $p \leq 0.01$.

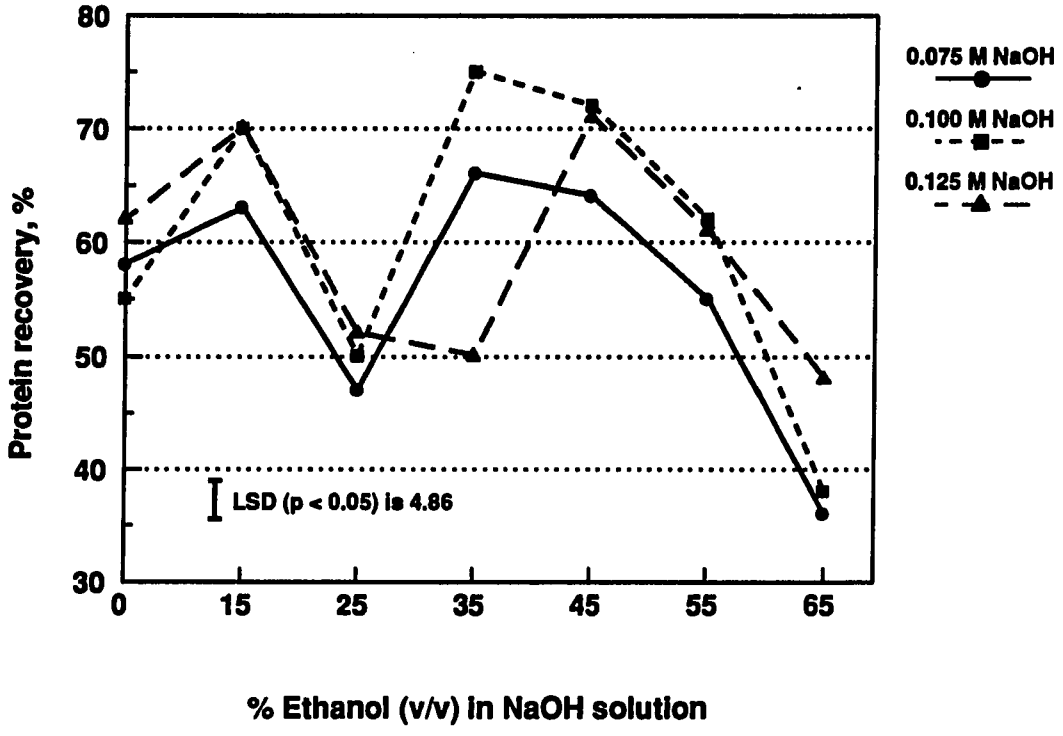


Figure 4. Effects of ethanol and NaOH concentrations on extraction of proteins from medium-hard dent corn (Pioneer 3732)

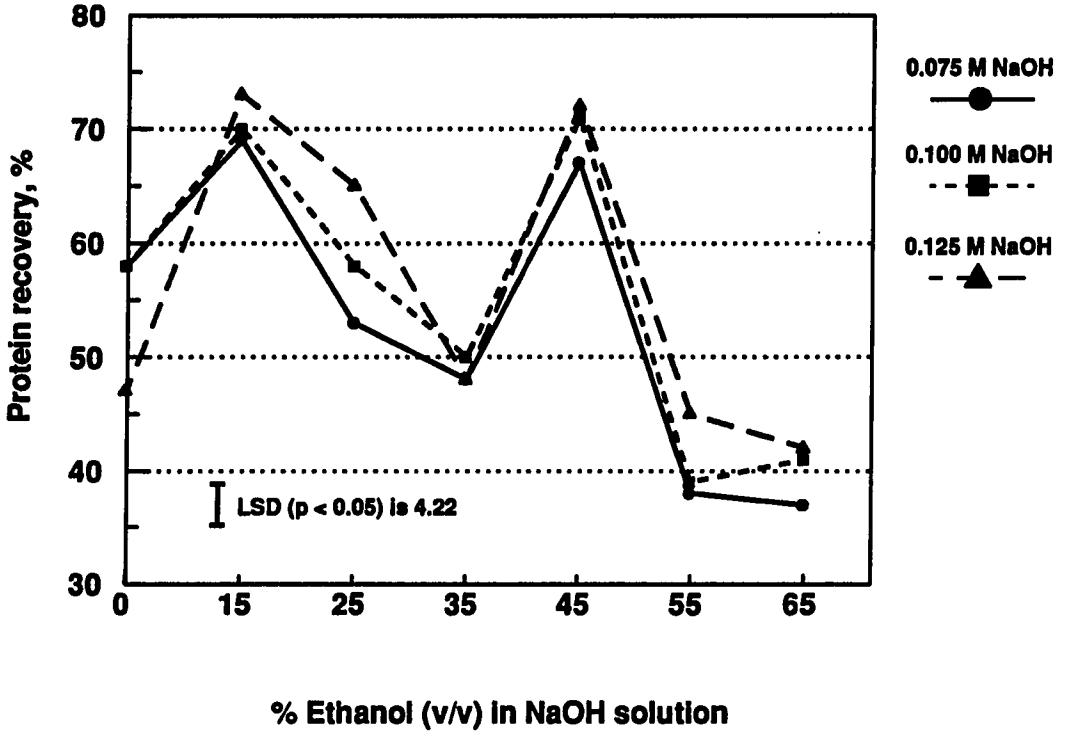


Figure 5. Effects of ethanol and NaOH concentrations on extraction of proteins from soft dent corn (Pioneer 3377)

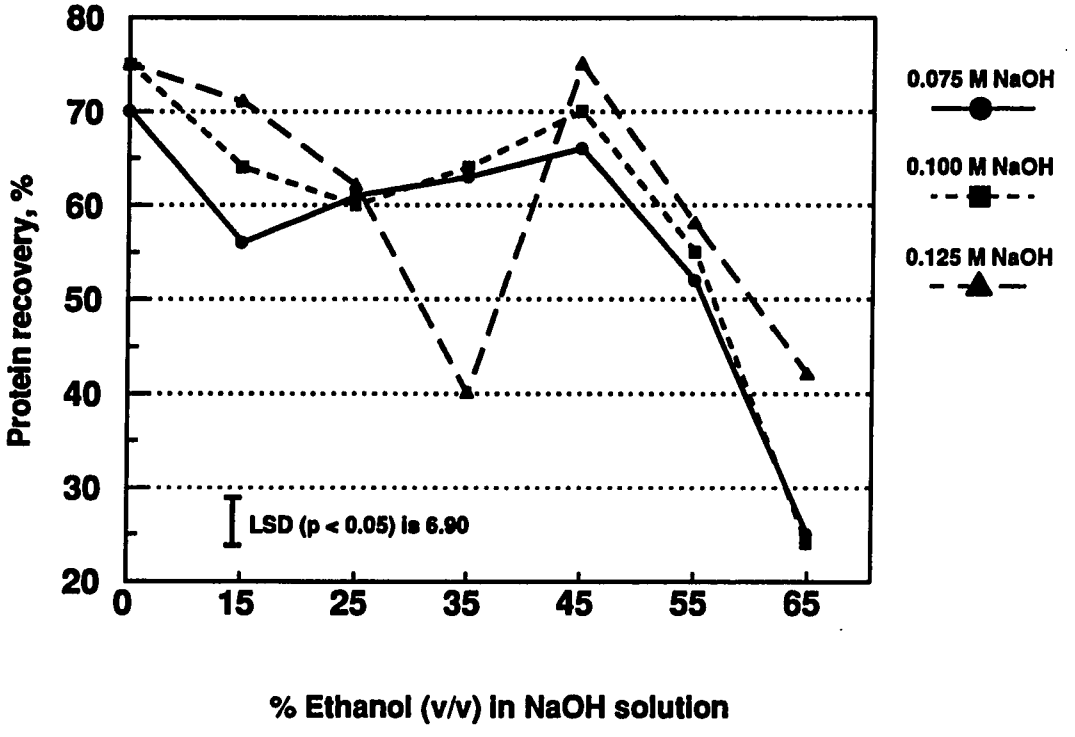


Figure 6. Effects of ethanol and NaOH concentrations on extraction of proteins from high-lysine corn

two classes of protein based on their solubility. It may be possible to maximize protein yields by extracting proteins at two alcohol concentrations. Ethanol should solubilize zein, and the aqueous alkali, the glutelins and perhaps some of the water-soluble proteins. Swallen (1941) reported a wide region of high zein yield for ethanol with the maximum at 60 to 65% alcohol concentration. Reiners et al. (1973a) observed the highest degree of zein solubility in 70/30 ethanol/water mixture. The study by Concon (1973) set concentration limits for ethanol at 15-25% of the total volume of the solvent while for NaOH, the limits were 0.10-0.12 N for vitreous endosperms and 0.05-0.08 N for floury endosperms. Our results, however, indicated that NaOH concentrations $\geq$ 0.1 M were needed to obtain high protein yields from both types of flaked, undegermed corn. Ethanol concentrations above 25% precipitated the glutelins (Concon, 1973). Thus, it is possible that mixtures containing less than 25% ethanol extracted mostly the glutelins and those containing more than 25% alcohol removed predominately zein. If this were the case, then the solubility of zein from defatted flaked whole corn differed markedly from previous studies which reported solubilities of proteins extracted from the corn endosperm (Russell and Tsao, 1982; Lusas et al., 1985; Concon, 1973).

The expected protein recovery from flaked whole corn defatted with 97.5% ethanol at 75^{0}C was estimated to be about 48% in Part II. Nearly all the ethanol:NaOH mixtures evaluated in this phase of the research had $\geq$ 48% protein recoveries from medium-hard dent corn, high-lysine corn and soft dent corn. The 65% ethanol:35% NaOH mixtures had protein recoveries from medium-hard dent corn and high-lysine corn which were significantly less than the expected 48%, while for soft dent corn, mixtures containing 55% ethanol recovered protein in significantly less quantities. The generally high protein recoveries from the ethanol:alkali mixtures were probably due to the higher protein extraction temperature employed (50^{0}C vs. 20^{0}C in Part II), the longer extraction time (2 hr), and the higher solvent:corn ratio (15 ml/g vs. 10 ml/g in Part II). From these

findings, the solvent selected for the succeeding stages of the protein extraction experiments was 45% ethanol:55% 0.100 M NaOH.

Optimization of Extraction Temperature

Increasing the temperature increased the amount of protein extracted (Table 3 and Figure 7). The protein recoveries for 45^{0}C and 25^{0}C were considerably less than those at 50^{0}C and 60^{0}C. No significant difference was detected between yields obtained at 50^{0}C and 60^{0}C. Protein solubility is enhanced by increasing temperature but only up to about 50^{0}C. Little is gained by using temperatures greater than 65^{0}C due in part to the increased denaturation at the higher temperatures. The optimum temperature selected was 55^{0}C.

Effects of Sonication

In the first set of trials, increasing the power level and the duration of sonication appeared to increase the extraction efficiency, but the yields were still less than that of the control (Table 4). The trends were not definitive (Figure 8a); thus, a second set of trials was performed at the maximum power level.

In the second trial, there was no significant difference between the protein yield of the control and corn samples sonicated for up to 10 sec. When the time was extended to more than 10 sec, the amount of protein extracted was significantly reduced (Figure 8b). These results were contrary to Lawhon's (1986) work on degerminated corn where he claimed sonication (20 KHz) increased protein yields. Ultrasonic waves are believed to destroy cellular structures (cell walls, membranes, and protein matrices) thereby loosening the protein and facilitating its extraction. Intense sound waves, however, can also cause the formation of bubbles in liquids due to the creation of alternating regions of compression and expansion, a phenomenon known as cavitation. During cavitation, the bubbles implode violently releasing vast amounts of energy within a very small area but

Table 3. Protein yields and recoveries from corn extracted with 45% ethanol:55% 0.100 M NaOH at different temperatures

Extraction temperature °C	Amount of protein extracted[1] (g/100 g corn, db, ffb)	Protein recovery[2] (%)
Pioneer 3732		
25	3.26 ± 0.49	36.7 ± 3.8[e]
45	5.27 ± 0.38	59.7 ± 4.8[b]
50	5.82 ± 0.22	71.9 ± 2.1[a]
60	6.52 ± 0.23	74.2 ± 8.6[a]
Pioneer 3377		
25	4.61 ± 0.50	49.1 ± 6.5[cd]
45	5.20 ± 0.23	55.4 ± 3.0[bc]
50	6.68 ± 0.10	71.2 ± 1.1[a]
60	6.93 ± 0.16	73.8 ± 1.9[a]
High-Lysine Corn		
25	3.95 ± 0.28	42.3 ± 5.1[de]
45	5.52 ± 0.25	59.0 ± 3.0[b]
50	6.55 ± 0.22	70.1 ± 3.1[a]
60	6.93 ± 0.16	74.2 ± 4.3[a]

[1]Initial crude protein contents were 8.83, 8.70 and 8.79 g/100 g corn, (dry basis, fat-free basis) for Pioneer 3732, Pioneer 3377 and high-lysine corn, respectively.

[2]Means with the same superscript are not significantly different at $p \leq 0.05$.

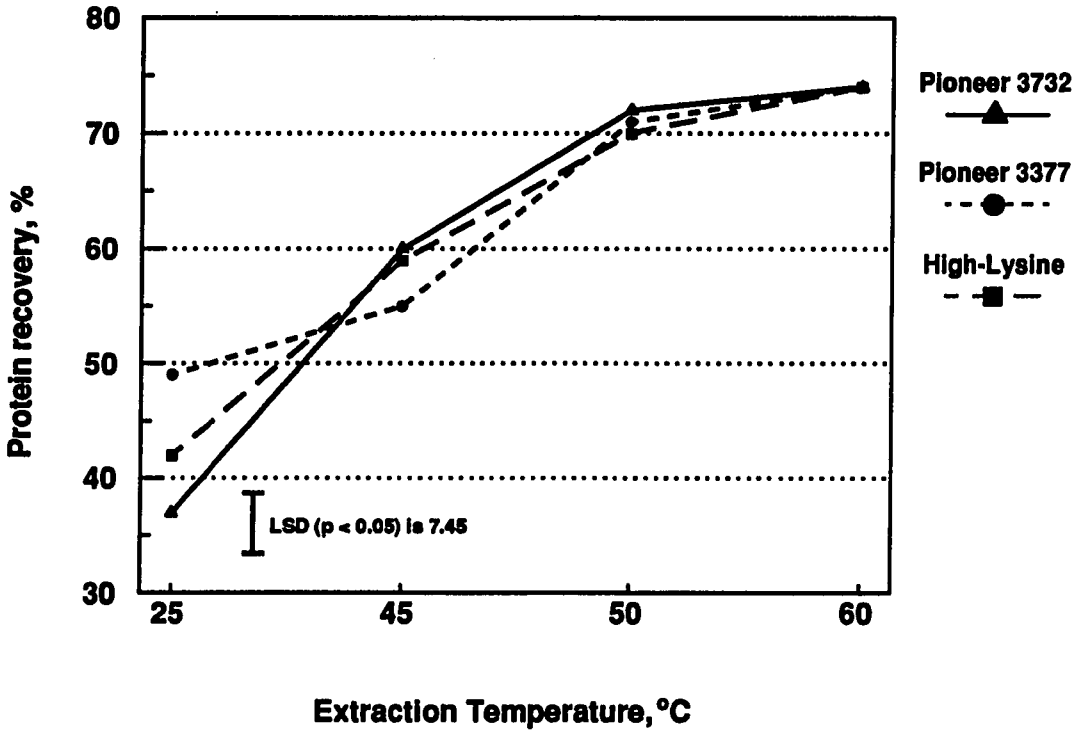


Figure 7. Effect of extraction temperature on protein recoveries from three corn varieties

Table 4. Effects of sonication on protein yields and recoveries from Pioneer 3732 extracted with 45% ethanol:55% 0.100 M NaOH at 55°C

Sonication treatment		Amount of protein extracted[1]	Protein recovery[2]
% Power	Time	(g/100 g corn, db, ffb)	(%)
Trial I			
0	0 (Control)	5.94 ± 0.31	69.4 ± 3.7[a]
100	10 sec	4.58 ± 0.35	53.9 ± 7.2[b]
100	1 min	5.62 ± 0.60	65.4 ± 3.8[a]
100	3 min	5.74 ± 0.60	67.0 ± 6.3[a]
75	10 sec	5.54 ± 0.59	64.5 ± 4.8[a]
75	1 min	3.87 ± 0.16	45.3 ± 3.9[cd]
75	3 min	4.58 ± 0.65	53.6 ± 8.1[b]
50	10 sec	3.35 ± 0.45	39.0 ± 4.1[d]
50	1 min	3.47 ± 0.17	40.5 ± 2.4[d]
50	3 min	4.07 ± 0.17	47.7 ± 4.6[bc]
Trial II			
0	0 (Control)	5.46 ± 0.15	66.2 ± 0.4[a]
100	1 sec	5.43 ± 0.37	65.9 ± 4.2[a]
100	10 sec	5.42 ± 0.44	65.7 ± 4.9[a]
100	5 min	2.87 ± 0.10	34.8 ± 1.1[b]

[1]Mean of three determinations. Db denotes dry basis, and ffb, fat-free basis.

[2]Based on the initial crude protein content of 8.58 g/100 g corn, db, ffb. Means with the same superscript are not significantly different at $p \leq 0.05$.

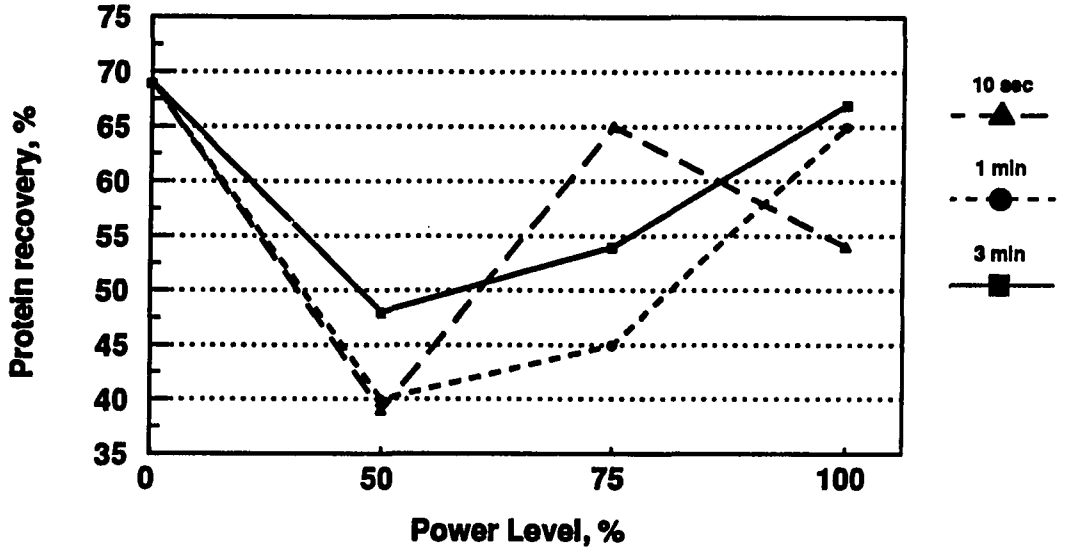


Figure 8a. Effects of sonication intensity and duration on the extraction of proteins from Pioneer 3732

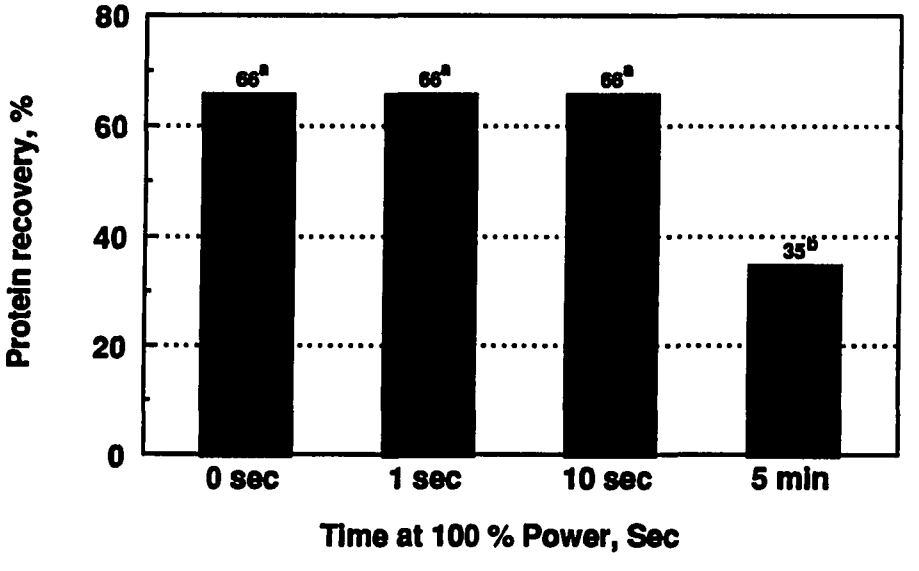


Figure 8b. Effect of time of sonication at 100% power on the extraction of proteins from Pioneer 3732

still capable of degrading organic compounds which may be nearby (Suslick, 1989). The cavitation phenomenon and/or insufficient sonication power [10 KHz, compared to 20 KHz used by Lawhon (1986)] may explain why sonication did not improve protein extractability in this study.

Effects of Homogenization

There was no significant difference between the amount of protein extracted from the control (unhomogenized) and the corn sample homogenized at 0.70 kg/mm^2 (1000 psi) (Table 5). Increasing the pressure to 3.16 kg/mm^2 (4500 psi) reduced the protein yield. Homogenization causes the rupture of structural components in the corn. Its action is believed to aid in loosening the protein from its matrix, allowing for easier extraction. Like the earlier sonication treatments, however, homogenization also did not enhance the extractability of corn proteins in our process.

Table 5. Effects of homogenization on protein yields and recoveries from corn extracted with 45% ethanol:55% 0.100 M NaOH at 55^{0}C

Homogenization treatment	Amount of protein extracted[1] (g/100 g corn, db, ffb)	Protein recovery[2] (%)
None (Control)	5.28 ± 0.12	61.5 ± 1.3^a
0.70 kg/mm^2 (1000 psi)	5.14 ± 0.10	59.8 ± 1.5^a
3.16 kg/mm^2 (4500 psi)	4.86 ± 0.06	55.6 ± 0.9^b

[1]Mean of 3 determinations. Db denotes dry basis, and ffb, fat-free basis.

[2]The initial crude protein content was 8.59 g/100 g corn, db, ffb. Means with the same superscript are not significantly different at $p \leq 0.05$.

SUMMARY AND CONCLUSIONS

Flaked undegermed corn was defatted using 97.5% ethanol and then protein-extracted using ethanol/alkali mixtures to verify the feasibility of sequentially recovering oil and protein from corn with ethanol. The results indicated that substantial quantities of oil and protein can be extracted using this process.

Medium-hard dent corn (Pioneer 3732) and soft dent corn (Pioneer 3377) exhibited maximum yields when extracted with either 45% or 15% (v/v) ethanol mixed with 0.100 M or 0.125 M NaOH. High-lysine corn showed high protein yields when extracted with 0.100 M or 0.125 M NaOH and with 45% ethanol:55% NaOH. The occurrence of high yields under two sets of solvent conditions strongly suggests the possibility of extracting two classes of corn proteins. It may be possible to maximize protein yields by employing a two-stage extraction process which utilizes two different alcohol concentrations. The 45% ethanol:55% 0.100 M NaOH mixture was selected as the optimum solvent for extracting protein from flaked undegermed corn.

Increasing the temperature from 25^{0}C to 60^{0}C increased the protein yields, with the maximum amount being obtained at 50^{0}C.

Neither sonication at 10 KHz nor homogenization significantly increased the amount of protein extracted. Lower extraction efficiencies were obtained during prolonged exposure to sonication or when higher homogenizing pressure was employed.

REFERENCES

AACC. 1983. Approved methods. 8th. ed. American Association of Cereal Chemists, Inc., St. Paul, MN.

Chen, L. F., and D. F. Houston. 1970. Solubilization and recovery of protein from defatted rice bran. Cereal Chem. 47:72-79.

Christianson, D. D., H. C. Nielsen, U. Khoo, J. M. Wolf, and J. S. Wall. 1969. Isolation and chemical composition of protein bodies and matrix proteins in corn endosperms. Cereal Chem. 46:372-381.

Christianson, D. D., U. Khoo, H. C. Nielsen, and J. S. Wall. 1974. Influence of *opaque-2* and *floury-2* genes on formation of proteins in particulates of corn endosperm. Plant Physiol. 53:851-855.

Concon, J. M. 1973. Rapid and simple method for the quantitative extraction of corn endosperm proteins. Anal. Biochem. 55:563-572.

Duvick, D. N. 1961. Protein granules of maize endosperm cells. Cereal Chem. 38:374-385.

Earle, F. R., J. J. Curtis, and J. E. Hubbard. 1946. Composition of the component parts of the corn kernel. Cereal Chem. 23:504-511.

Fellers, D. A., V. Sinkey, A. D. Shepherd, and J. W. Pence. 1966. Solubilization and recovery of protein from wheat millfeeds. Cereal Chem. 43:1-13.

Hamilton, T. S., B. C. Hamilton, B. C. Johnson, and H. H. Mitchell. 1951. The dependence of the physical and chemical composition of the corn kernel on soil fertility and cropping systems. Cereal Chem. 28:163-176.

Hassanen, N. Z., L. A. Johnson, J. T. Farnsworth, and E. W. Lusas. 1985. Sequential extraction process for extracting oil and aflatoxin from cottonseed. Abstract no. 81. J. Am. Oil Chem. Soc. 62(4):639.

Hinton, J. J. C. 1953. The distribution of protein in the maize kernel in comparison with that of wheat. Cereal Chem. 30:441-445.

Huster, H., F. Meuser, and C. Hoepke. 1983. Method of producing starch from grain or ground grain products by the wet process. U. S. Patent No. 4,416,701.

Khavkin, E. E., S. I. Misharin, Y. Y. Markov, and A. A. Peshkova. 1978. Identification of embryonal antigens of maize: Globulins as primary reserve proteins of the embryo. Planta 143:11-20.

Landry, J., and T. Moureaux. 1970. Heterogeneite des glutelines du grain de mais: Extraction selective et composition en acides amines des trois fractions isolees. Bull. Soc. Chim. Biol. 52:1021-1037.

Landry, J., and T. Moureaux. 1980. Distribution and amino acid composition of protein groups located in different histological parts of maize grain. J. Agric. Food Chem. 28:1186-1191.

Landry, J., and T. Moureaux. 1981. Physicochemical properties of maize glutelins as influenced by their isolation conditions. J. Agric. Food Chem. 29:1205-1212.

Landry, J., J. W. Paulis, and D. A. Fey. 1983. Relationship between alcohol-soluble proteins extracted from maize endosperm by different methods. J. Agric. Food Chem. 31:1317-1322.

Lawhon, J. T. 1986. Process for recovery of protein from agricultural commodities prior to alcohol production. U. S. Patent No. 4,624,805.

Lusas, E. W., K. C. Rhee, and J. T. Lawhon. 1985. Recovery and utilization potential of proteins from corn and other cereals in alcohol production. International Conference on Ethanol from Biomass. Phillipsburg, Netherlands Antilles.

Meuser, F., and H. German. 1984. Use of high-pressure homogenization for the production of starch from maize. Starch 36:116-121.

Nielsen, H. C., J. W. Paulis, C. James, and J. S. Wall. 1970. Extraction and structure studies on corn glutelin proteins. Cereal Chem. 47:501-511.

Osborne, T. B., and L. B. Mendel. 1914. Nutritive properties of the maize kernel. J. Biol. Chem. 18:1-16.

Paulis, J. W. 1982. Recent developments in corn protein research. J. Agric. Food Chem. 30:14-20.

Paulis, J. W., and J. S. Wall. 1969. Albumins and globulins in extracts of corn grain parts. Cereal Chem. 46:265-273.

Reiners, R. A., J. S. Wall, and G. E. Inglett. 1973a. Corn proteins: Potential for their industrial use. Pages 285-301 in Y. Pomeranz, ed. Industrial uses of cereals. American Association of Cereal Chemists, Inc., St. Paul, MN.

Russell, M. H., and G. T. Tsao. 1982. Protein separation from corn endosperm by solvent extraction. AIChE Symp. Ser. 78:83-89.

SAS. 1987. SAS/STAT guide for personal computers. Version 6 ed. SAS Inst., Inc., Cary, NC.

Suslick, K. 1989. The chemical effects of ultrasound. Scientific American 260(2):80-86.

Swallen, L. C. 1941. Zein - a new industrial protein. Ind. Eng. Chem. 33(3):394-398.

Turner, J. E., J. A. Boundy, and R. J. Dimler. 1965. Zein: A heterogeneous protein containing disulfide-linked aggregates. Cereal Chem. 42:452-461.

Wall, J. S., and J. W. Paulis. 1978. Corn and sorghum grain proteins. Pages 135-163 in Y. Pomeranz, ed. Advances in cereal science and technology. Volume II. American Association of Cereal Chemists, Inc., St. Paul, MN.

Wilson, C. M. 1987. Proteins of the kernel. Pages 273-297 in S. A. Watson and P. E. Ramstad, eds. Corn: Chemistry and technology. American Association of Cereal Chemists, Inc., St Paul, MN.

Wolf, M. J., U. Khoo, and H. L. Seckinger. 1969. Distribution and subcellular structure of endosperm protein in varieties of ordinary and high-lysine maize. Cereal Chem. 46:253-263.

Wu, Y. V. and K. R. Sexson. 1976. Protein concentrate from normal and high-lysine corns by alkaline extraction: Preparation. J. Food Sci. 41:509-511.

PART IV. SIMULTANEOUS DRYING OF ETHANOL AND EXTRACTION OF CRUDE OIL FROM DRIED FLAKED UNDEGERMED CORN

ABSTRACT

The feasibility of a processing operation which simultaneously dehydrates ethanol and extracts crude oil from dried, flaked, undegermed corn was studied using a simulated countercurrent extraction system. The moisture adsorption capacity of the flake bed was 26 g/kg corn (initially, < 2% M.C.) which was sufficient to dehydrate 35g of 95% ethanol/100 g corn (2.5 gals/bu) at 2% moisture to 99% ethanol. This ethanol (at 75^{o}C) extracted 93% of the available crude oil in the corn, demonstrating the viability of this phase of the process.

INTRODUCTION

Ethanol Production and Utilization

Ethanol is produced from grains or biomass by anaerobic fermentation of saccharified starch using the yeast *Saccharomyces cerevisiae*. The broth contains 6-12% alcohol together with small amounts of aldehydes, ketones, and methanol. Volatile alcohol components are separated from the fermented mash (beer) by distillation. Still designs vary to match the selected type and quality of ethanol distillates. Beer-stills produce 110-160^0 proof distillates which could be fed to multiple-column stills to produce 190^0 proof (95% w/w) ethanol. At this concentration, water and ethanol form a constant boiling azeotrope which can be broken by adding benzene or diethyl ether in order to obtain anhydrous ethanol. Ethanol can then be distilled from this mixture, leaving the other two components behind. The product is 99.9% ethanol but this second distillation adds an additional 1950-2228 KJ/l (7,000-8,000 BTU/gal) to the 5571 KJ/l (20,000 BTU/gal) consumed in the production of 95% ethanol (Maisch, 1987).

Aside from being used in beverages or industrial solvents, anhydrous ethanol can be a source of liquid fuel when blended with gasoline. Ethanol has also been evaluated as a solvent for the extraction of corn lipids and other vegetable oils (Beckel et al., 1948; Rao and Arnold, 1956; Karnofsky, 1981; Hassanen et al., 1985). These applications show that fermentation alcohol has the ability to reduce the United States' dependence on foreign petroleum-based products. However, the potential of ethanol utilization has not been fully exploited partly because of the extensive energy requirements of the distillation procedure. It has been reported that distillation to water-free alcohol could consume from 50-80% of the total energy used in a typical ethanol manufacturing plant (Hong et al., 1982; Ladisch and Tsao, 1982).

Alternative Processes for Ethanol Production

A process was developed by Ladisch and Tsao (1982) for energy-efficient recovery of anhydrous ethanol. The method involves partial distillation of 12% alcohol to a 70-90% aqueous product followed by water adsorption using cellulose, cellulose derivatives, corn residue or cracked corn. Ladisch et al. (1984) designed a pilot-scale adsorber which utilized cornmeal to dehydrate ethanol vapors. It was suggested that the cornmeal could later be used to make fermentation-derived ethanol after its adsorbing capacity was exhausted. Earlier studies by Chung and Pfost (1967) evaluated corn hull, corn gluten, corn germ and corn starch for their ability to adsorb and desorb water vapor, and determined moisture-vapor isotherms. Gupta and Bhatia (1969) carried out sorption-desorption studies of water, methanol, ethanol, and carbon tetrachloride vapors on starch. Ethanol was observed to adsorb at a slower rate and to a smaller extent than water at 35^{0}C. Other biomass materials which have been screened for ethanol dehydration potential include cellulose, xylan, corn residue, corn and potato starches, wheat straw and bagasse (Hong et al., 1982).

Anhydrous ethanol is the preferred solvent for oils extraction because a moisture content of less than 1% is necessary to achieve complete miscibility between corn oil and the alcohol at 70^{0}C (Rao and Arnold, 1956, 1957). However, its cost is considerably more expensive than the 95% (w/w) azeotrope. Thus, the use of anhydrous ethanol for corn oil recovery may not be economically viable unless it can be generated during the extraction step. Based on this premise, Chien et al. (1988) claimed to be able to simultaneously dehydrate 95% ethanol and extract crude oil from ground corn at 68^{0}C. The moisture adsorption capacity was reported to be 32 g/kg dried ground corn using 95% ethanol while the amount of crude oil extracted was 45 g/kg dried ground corn.

Objective of the Study

This investigation was undertaken to determine the feasibility of utilizing a countercurrent system to extract crude oil from dried, flaked, undegermed corn using ethanol while simultaneously removing moisture from the alcohol.

MATERIALS AND METHODS

Sample Preparation

Twenty-five batches of medium-hard dent corn (Pioneer 3732) weighing 350 g each were prepared. Each batch was cracked and then flaked using the Roskamp rollermill (Model K, Roskamp Mfg., Inc., Waterloo, IA). The flaked corn was placed in aluminum pans and dried at 75^{0}C in a forced-air convection oven to a moisture content of $\leq$ 2%. Each sample was stored in labeled resealable polyethylene bags (2.7 mils thickness) and kept in a desiccator until used. All batches were analyzed for initial crude free fat using AACC standard procedure 30-20 (AACC, 1983) and for initial moisture content by Karl Fischer titration using ASTM standard method E 203-75 (ASTM, 1975).

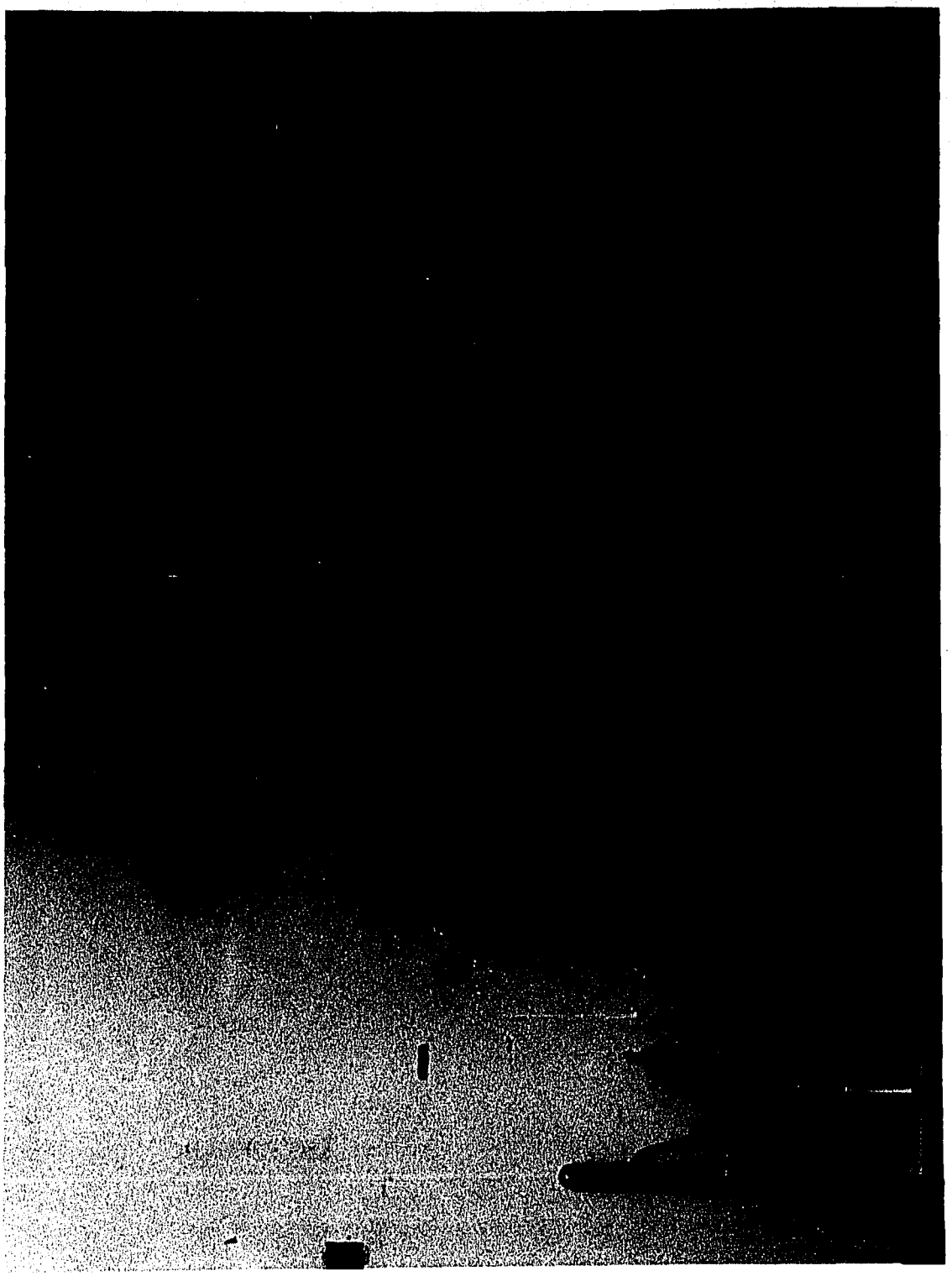
Solvent Preparation for Extraction Stages

The ethanol concentrations of the seven extraction stages to be used for start-up of the extraction process were based upon: a) the exponential relationship between oil extractability and alcohol concentration; b) the assumption that the amount of ethanol retained in the marc (solvent-laden defatted flakes) is 65% of the weight of the corn; and c) the amount of ethanol produced from the fermentation of one bushel of corn (15% moisture content), which is 2.5 gallons, or 35 g ethanol/100 g corn at 3% moisture content. These concentrations ranged from 97.2% (v/v) to 99.5%. The water content was measured by Karl Fischer titration (ASTM, 1975).

Countercurrent Extraction System

The oil extraction system (Figure 1) consisted of jacketed glass vessels covered with rubber stoppers (A), 95% ethanol (B), solvents/miscellas for extraction (C), and full miscellas (D) for oil recovery in the rotary evaporator (E). Solvent temperatures were monitored by thermometers inserted through the stoppers. Evaporation of the solvents

Figure 1. The laboratory countercurrent extraction system



was minimized by cold water condensers (F) attached to the stoppers. Contamination with atmospheric moisture was eliminated by flushing the system before every extraction trial with nitrogen gas (G) (which passed through a desiccant (H) before entering the system), and by attaching tubes with desiccants (I) to every condenser and other outlets. A water bath (J) supplied the hot water which was circulated through the glass vessels by a centrifugal pump (K). Solvent circulation through the corn was accomplished by the diaphragm pump (L). A peristaltic pump (M) recovered the ethanol obtained by rotary evaporation of the miscella into the graduated separatory funnel (N).

Countercurrent Oil Extraction and Ethanol Dehydration

Six hundred ml of each solvent was placed in the appropriate jacketed glass vessel. This amount was sufficient for a 2:1 solvent:corn (w:w) ratio. The stoppers were replaced and heated circulating water was used to pre-heat and maintain the temperature of the system at 75^{0}C. The dried, flaked corn was placed in the extraction vessel and subjected to seven extraction stages. In each stage, the solvent was circulated through the flakes for 10 mins. Except for the first extraction vessel, the contents of each vessel were pumped into the previously emptied container after circulation, thus advancing solvent flow. The bed was then allowed to drain by gravity for 5 min. After the first stage, the miscella was drained into the recovery vessel and drawn by vacuum into the rotary evaporator. The alcohol was evaporated, recovered by condensation, and pumped into a graduated separatory funnel. The volume of dried ethanol was carefully measured to correspond to the specified weight for mixing with 95% ethanol, producing a fresh preparation of 97.2% ethanol in vessel number 7. The remaining amount of the condensed, dry ethanol in the graduated separatory funnel was emptied into a labelled screw-capped glass vial and stored in a desiccator for moisture analysis. The extraction vessel was disconnected from the system and a small amount of the defatted flakes was placed in a screw-capped vial which was also stored in a desiccator for moisture analysis.

The remaining flakes were removed from the vessel, air-dried and stored in resealable polyethylene bags for further analysis. The sample flask from the rotary evaporator was disconnected and set aside for oil recovery and yield determination. The cleaned extraction vessel and a new sample flask were then replaced in the system for the next succeeding extraction. The procedure was repeated for 19 more extraction sequences, where the first 14 were used to establish equilibrium. Starting on the tenth run, a portion of the marc was subjected to rotary evaporation to recover the condensate. The details of the extraction sequences are presented in Figure 2.

Analyses of Samples

The Karl Fischer titration method (ASTM, 1975) was used to determine the moisture content of the defatted flakes immediately after extraction, of the ethanol recovered from the full miscella, of the condensate from the marc, and of all the miscellas after the final extraction sequence. The oil yield was determined for each run by extracting the oil and solids from the miscella with petroleum ether, filtering the washings into a pre-weighed flask and evaporating the solvent in a water bath. The solids were air-dried and their amounts were recorded. The amounts of oil in the miscellas were also determined after the final run. All determinations were performed in triplicate except for the yields of oil and solid residues.

Statistical Analyses

Data were analyzed using a Statistical Analysis System program (SAS, 1987). Significant differences among extraction runs and paired comparisons were detected by the test for Least Significant Difference (LSD). Probability levels of $p \leq 0.05$ were deemed significant.

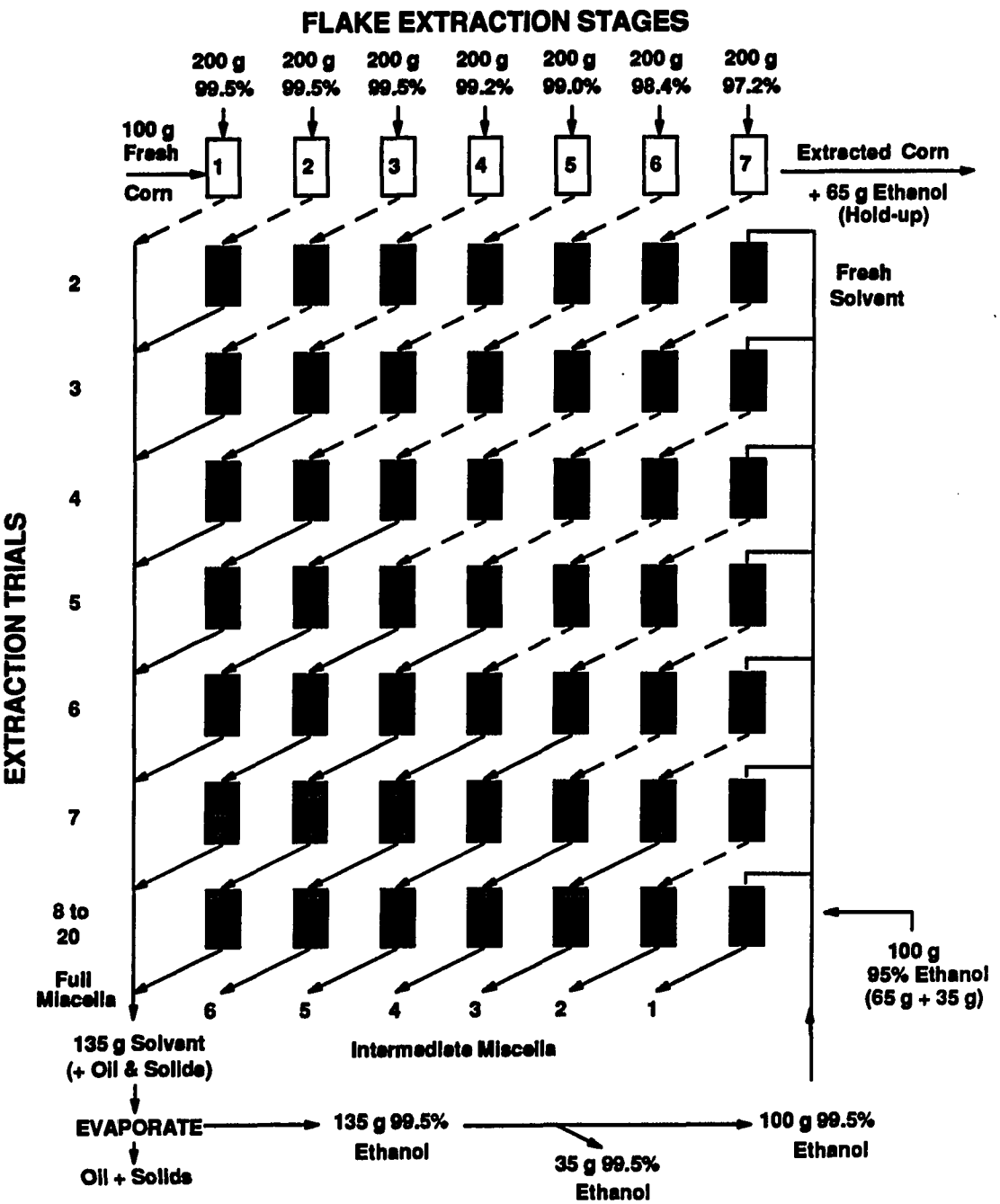


Figure 2. Flow scheme of the extraction procedure

RESULTS AND DISCUSSION

Establishment of System Equilibrium

The system was considered to be at steady-state when near-constant yields of oil and ethanol-soluble solids, and near-constant moisture contents in corn or dried ethanol were obtained during extraction. Steady-state conditions were achieved after the fourteenth extraction sequence (Appendix Tables A-6, A-7, and A-8). No significant differences were observed in the moisture contents of the marc (solvent-laden defatted flakes) or dried ethanol from the fifteenth to the twentieth run (Appendix Tables A-10 and A-11), thus verifying that the system was already at steady-state or equilibrium. The data for the last six extraction trials were used for data collection.

Ethanol Dehydration

The significant increase in moisture content observed in the flaked corn during oil extraction and the substantial reduction in the amount of water in the ethanol (Table 1) indicated that drying of the alcohol occurred. The moisture adsorption capacity of the flaked undegermed corn (initially at < 2% M.C.) was calculated to be nearly 26 g/kg of corn which was sufficient to dehydrate 2.5 gal of 95% ethanol to about 99% ethanol for each bushel extracted. This is the amount of ethanol produced from fermenting one bushel of corn. The water content of the ethanol obtained from the marc verified the assumption that the solvent held up in the flake bed was approximately 95% ethanol (Appendix Table A-8). The mass balance on water content (Table 2) showed good agreement.

Oil Extraction

The mean initial crude free fat content of flaked Pioneer 3732 was 4.88% (db). The mean oil yield from the full miscellas of the last 6 extraction trials was 4.52% (db)

Table 1. Moisture content of corn flakes before and after oil extraction (marc) and of the ethanol recovered from miscella evaporation

Extraction run	Moisture content, %[1]			
	Flaked corn		Ethanol	
	Before extraction	After extraction	From miscella	From marc
15	1.18	3.61	1.11	4.74
16	1.12	3.54	1.13	4.55
17	1.17	3.84	1.11	4.79
18	1.11	3.81	1.10	4.93
19	1.12	3.68	1.09	5.05
20	1.04	3.68	1.12	5.02
Grand mean[2]	1.12[a]	3.69[b]	1.12[**]	4.85

[1]Weight basis for corn, volume basis for ethanol.

[2]Means with the same superscript are not significantly different at $p \leq 0.05$.

[**]Significantly different from 95% and 97.2% ethanol at $p \leq 0.01$.

(Appendix Table A-6). The oil recovery efficiency of the countercurrent extractor was 92.6%.

Table 3 shows the profile of oil concentrations in each stage of extraction after the last run was completed. The bulk of the oil was extracted in the first 3 stages where the miscellas are more anhydrous than those from the latter stages. As the aqueous concentration of ethanol increases, there is a corresponding increase in its polarity which reduces the alcohol's oil extraction capability. At 65°C, corn oil and anhydrous ethanol are miscible but only 20% oil is soluble in 95% ethanol at 78°C.

Table 2. Water balance during oil extraction

Moisture in:						
Run number	Wt. corn g	M.C.[a] of corn, %	Weight water in corn, g	M.C. of 95% ethanol, % wt. basis	Weight water in 232 g 95% ethanol, g	Total water in, g
15	228.9	1.18	2.70	6.76	15.67	18.37
16	231.0	1.12	2.59	6.76	15.67	18.26
17	227.8	1.17	2.66	6.76	15.67	18.33
18	231.4	1.11	2.57	6.76	15.67	18.24
19	227.9	1.12	2.55	6.76	15.67	18.22
20	231.3	1.04	2.41	6.76	15.67	18.08
Moisture out:						
Run number	Wt. marc g	M.C. of marc, %	Weight water in marc, g	M.C. of recovered ethanol, % wt. basis	Weight water in recovered ethanol, g	Total water out, g
15	374.6	3.61	13.52	1.42	3.89	17.41
16	377.5	3.54	13.36	1.45	3.91	17.27
17	372.6	3.84	14.31	1.41	3.84	18.15
18	378.0	3.81	14.40	1.41	3.90	18.30
19	366.0	3.68	13.47	1.40	3.79	17.26
20	364.8	3.63	13.24	1.42	3.84	17.08

[a]M.C. denotes moisture content.

Table 3. Oil and moisture concentration profiles in extraction stages

Stage number	Oil per 100 g miscella, g	Oil from 100 g dry corn, g[a]	M.C.[b] % by volume
1	2.42	4.83	1.55
2	1.88	3.76	1.52
3	0.93	1.86	1.52
4	0.50	0.99	1.58
5	0.37	0.73	1.61
6	0.27	0.55	1.66
7	0.24	0.49	2.76

[a]Calculated by multiplying the amount of oil per 100 g miscella by 2, following the 2:1 miscella:flake (w:w) ratio.

[b]M.C. denotes moisture content.

SUMMARY AND CONCLUSIONS

The moisture adsorption capacity of the flaked whole corn (< 2% M.C.) was 26 g/kg of corn. This capacity was sufficient to dehydrate 35 g of 95% ethanol/100 g corn initially at 2% moisture (2.5 gallons ethanol per bushel) to 99% ethanol. The oil extraction efficiency of the dry ethanol at 75^{o}C was 93%, leaving 0.36% (db) residual oil. It is possible to simultaneously extract the oil from corn and dehydrate 95% ethanol to about 99% ethanol in countercurrent extraction of dried, flaked, undegermed corn using a 2:1 solvent:flake (w:w) ratio.

REFERENCES

AACC. 1983. Approved methods. 8th ed. American Association of Cereal Chemists Inc., St. Paul, MN.

ASTM. 1975. Standard test method for water using Karl Fischer reagent. ASTM designation: E 203-75 (Reapproved 1986). American Society for Testing and Materials, Philadelphia, PA.

ASTM. 1985. Standard test method for water in organic liquids by coulometric Karl Fischer titration. ASTM designation: E 1064-85. American Society for Testing and Materials, Philadelphia, PA.

Beckel, A. C., P. A. Belter, and A. K. Smith. 1948. The non-distillation alcohol extraction process for soybean oil. J. Am. Oil Chem. Soc. 25:10-11.

Chien, J. T., J. E. Hoff, and L. F. Chen. 1988. Simultaneous dehydration of 95% ethanol and extraction of crude oil from dried ground corn. Cereal Chem. 65(6):484-485.

Chung, D. S., and H. B. Pfost. 1967. Adsorption and desorption of water vapor by cereal grains and their products. Part I: Heat and free energy changes of adsorption and desorption. Trans. of the ASAE 10:549-557.

Gupta, S. L., and R. K. S. Bhatia. 1969. Sorption of water and organic vapours on starch at 35°C. Indian J. of Chem. 7:1231-1233.

Hassanen, N. Z., L. A. Johnson, J. T. Farnsworth, and E. W. Lusas. 1985. Sequential extraction process for extracting oil and aflatoxin from cottonseed. Abstract no. 81. J. Am. Oil Chem. Soc. 62(4):639.

Hong, J., M. R. Ladisch, and G. T. Tsao. 1981. Vapor-liquid equilibria of the water-ethanol system at low alcohol concentrations. J. Chem. Eng. Data 26:307-309.

Hong, J., M. Voloch, M. R. Ladisch, and G. T. Tsao. 1982. Adsorption of ethanol-water mixtures by biomass materials. Biotechnology and bioengineering 24(3):725-730.

Karnofsky, G. B. 1981. Ethanol and isopropanol as a solvent for full-fat cottonseed extraction. Oil Mill Gaz. 85:34.

Ladisch, M. R., and G. T. Tsao. 1982. Vapor phase dehydration of aqueous alcohol mixtures. United States Patent No. 4,345,973.

Ladisch, M. R., M. Voloch, J. Hong, P. Blenkowskl, and G. T. Tsao. 1984. Cornmeal adsorber for dehydrating ethanol vapors. Ind. Eng. Chem. Process Des. Dev. 23:437-443.

Maisch, W. F. 1987. Fermentation processes and products. Pages 553-574 in S. A. Watson and P. E. Ramstad, eds. Corn: Chemistry and technology. American Association of Cereal Chemists, Inc., St. Paul, MN.

Rao, R. K., and L. K. Arnold. 1956. Alcoholic extraction of vegetable oils. II. Solubilities of corn, linseed and tung oils in aqueous ethanol. J. Am. Oil Chem. Soc. 33:82-84.

Rao, R. K., and L. K. Arnold. 1957. Alcoholic extraction of vegetable oils. IV. Solubilities of vegetable oils in aqueous 2-propanol. J. Am. Oil Chem. Soc. 34:401-404.

SAS. 1987. SAS/STAT guide for personal computers. Version 6 ed. SAS Inst. Inc., Cary, NC.

PART V. INTEGRATING ELEMENTS OF SEQUENTIAL EXTRACTION PROCESSING OF FLAKED WHOLE CORN USING ETHANOL

ABSTRACT

A radical new approach to fractionating dried, flaked corn was studied. The countercurrent process involved the sequential extraction of crude oil and simultaneous dehydration of ethanol. Protein was extracted using a mixture of alkali and ethanol. The procedure provided a means of recycling the alcohol from ethanol fermentation to upstream steps of extraction. Ethanol was able to extract 90% and 94% of the oil from medium-hard dent corn (Pioneer 3732) and high-lysine corn, respectively. These recoveries were significantly greater than the 72% estimated for recovery by wet milling corn and prepress hexane-extraction of the germ. The moisture adsorption capacities of the flaked whole corn (initially at < 2% M.C.) were 20 g/kg dent corn and 18 g/kg high-lysine corn. These capacities were sufficient to dry 35 g of 95.0% ethanol/100 g corn initially at < 2% M.C. (2.5 gal/bu) to 99.0% ethanol. The alcohol-alkali mixture removed as much as 65% of the available corn protein. The freeze-dried protein extract from medium-hard dent corn (Pioneer 3732) contained 72.5% crude protein (db). The variety of corn used did not significantly affect the oil and protein yields. The sequential extraction of corn with ethanol appears to be technically feasible and may have considerable economic potential in industries which produce fuel ethanol by cornstarch fermentation.

INTRODUCTION

Significance of the Process

Wet grain milling is used to recover starch from corn and this process has not changed significantly over the last 50 years. Cornstarch is used in the manufacture of high-fructose corn syrups (HFCS), and for fermentation into industrial solvents and fuel ethanol. Wet-milling techniques are preferred to dry milling because the starch is recovered in greater yield and purity. However, current wet-milling methods use vast amounts of energy, capital, and water. These factors have impeded the expansion of the wet milling industry brought about by the increased demand for fuel ethanol and HFCS. In addition, the traditional feed markets are becoming saturated with the by-products from wet corn mills, resulting in lower prices for corn gluten meal, corn gluten feed, and corn germ meal.

More cost-effective methods to process corn into starch and starch-derived products are necessary if these and related industries are to remain competitive and expand. This can be achieved by reducing operating costs for processing, increasing yields of high-value products, and upgrading the value of by-products. The by-products of today's wet corn mills are produced in a manner which makes them suitable only for feed, despite the fact that corn proteins possess properties which have potential use in the food industry. The Sequential Extraction Process (Figure 1) is a radical new approach to corn milling which hopes to accomplish the above goals and contribute to the expansion of the industry. It has three novel steps: 1) simultaneous extraction of corn oil and drying of the alcohol; 2) use of alcohol/alkali to extract protein and produce a food-grade protein concentrate; and, 3) recycling of ethanol from fermentation of cornstarch to upstream extraction steps. Earlier studies have determined the feasibility of each of these steps using the corn germ for oil recovery and corn endosperm for protein extraction.

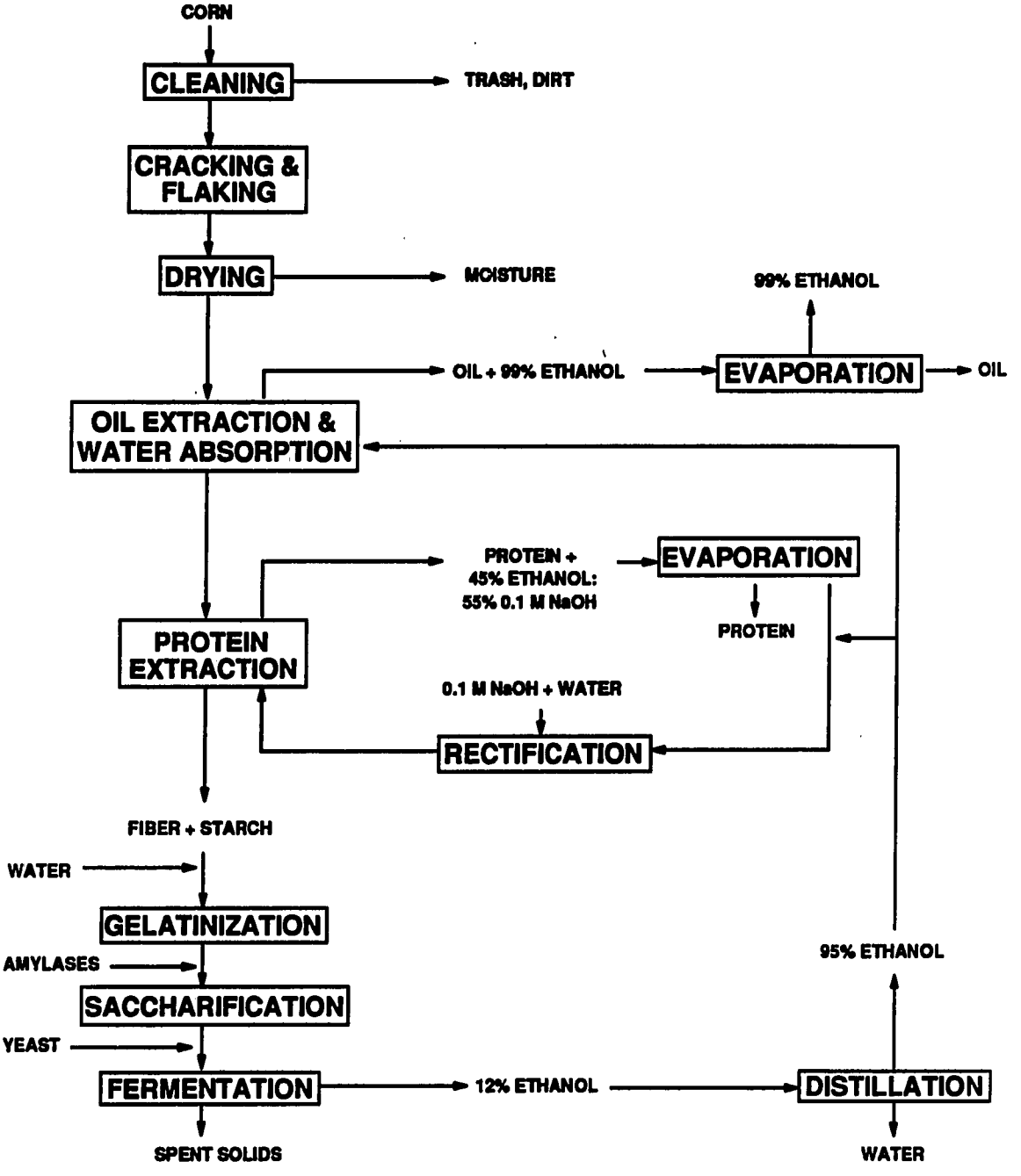


Figure 1. Sequential extraction processing of corn

Oil extraction using alcohols Prior art in using alcohols to extract corn components is limited. Beckel et al. (1948) developed a non-distillation extraction process using ethanol to recover soybean oil. Rao et al. (1955) and Rao and Arnold (1956a, 1956b) studied the solubilities of 13 common vegetable oils in aqueous ethanol and reported that a moisture content of less than 1% in the alcohol was necessary to achieve complete miscibility between corn oil and the alcohol at 70°C. More recently, sequential extraction processes using ethanol to extract oil and aflatoxin from cottonseed were developed (Hassanen et al., 1985; Karnofsky, 1981).

Alcohol dehydration Ladisch and Tsao (1982) developed an energy-efficient recovery process for anhydrous ethanol which involved the partial distillation of 12% alcohol to a 70-90% aqueous product followed by adsorption of water using cellulose, corn residue or cracked corn. Ladisch et al. (1984) designed a pilot scale adsorber which utilized corn meal to dry ethanol vapors. Chien et al. (1988) reported on a column extraction process which simultaneously dehydrated 95% ethanol and extracted crude oil from dried ground corn at 68°C.

Protein extraction using ethanol Substantial amounts of zein are soluble in alcohols and can be extracted with aqueous ethanol (Swallen, 1941). Paulis (1982) and Landry et al. (1983) utilized ethanol combined with salts or reducing agents to separate glutelins. The optimum conditions for extracting corn endosperm proteins with ethanol were concentrations ranging from 55-70% (Russell and Tsao, 1982; Turner et al., 1965) and temperatures close to 25°C (Chen and Houston, 1970; Concon, 1973; Turner et al., 1965). Russell (1980) reported total protein recoveries of 80% from corn endosperm using a process which combined elements of dry milling to separate fiber and germ followed by extraction with ethanol and then alkali to remove zein and glutelin, respectively. Lusas et al. (1985) reported that extraction efficiency from degermed corn can be as much as 85% with proper pH adjustment of the aqueous phase. Lawhon (1986) reported that sonication improved protein yields from degermed corn.

Research Objectives

This study was undertaken to evaluate the feasibility of a sequential extraction approach to corn milling using ethanol, first to extract oil while simultaneously dehydrating the alcohol, and then to remove the proteins from the other corn components. The specific objective was to verify if the elements studied separately in the previous sections could be integrated into a single continuous process.

MATERIALS AND METHODS

Preparation of Corn

Medium-hard dent corn (Pioneer 3732, Dept. of Agricultural Engineering Grain Quality Laboratory, Iowa State University, Ames, IA) and high-lysine corn (Crow's Hybrid Seed Co., Milford, IL) were used in this study. Twenty-five batches, each weighing 350 g, were prepared for each corn variety. Each batch was cracked then flaked using the Roskamp rollermill (Model K, Roskamp Mfg., Inc., Waterloo, IA). The flaked corn samples were placed in aluminum pans and dried at 50^{0}C in a forced-air convection oven to a moisture content of < 2%. Each dried sample was stored in a labeled resealable polyethylene bag (2.7 mils thickness) and kept in a desiccator until used.

Solvent Preparation

Fifteen extraction trials were completed to obtain miscellas which were at steady-state. The seven ethanol concentrations for start-up of the countercurrent extraction process were determined in Part IV and ranged from 97.2% to 99.5% (v/v).

Sequential Extraction Processing of Corn

The oil extraction system (Figure 2) was modified from the laboratory extractor-simulator used by Hassanen et al. (1985) by using multiple solvent holding vessels for the seven ethanol concentrations. Dried nitrogen gas was flushed through the system to prevent moisture contamination from the atmosphere. Desiccants were attached to the condensers to prevent entry of atmospheric moisture in the vessels. The rotary evaporator was incorporated in the system to separate dry ethanol and oil from the miscella without exposure to air. A diaphragm pump was used to circulate the solvent through the heat exchanger and the flaked corn bed. A peristaltic pump brought up the ethanol from the rotary evaporator into the graduated separatory funnel.

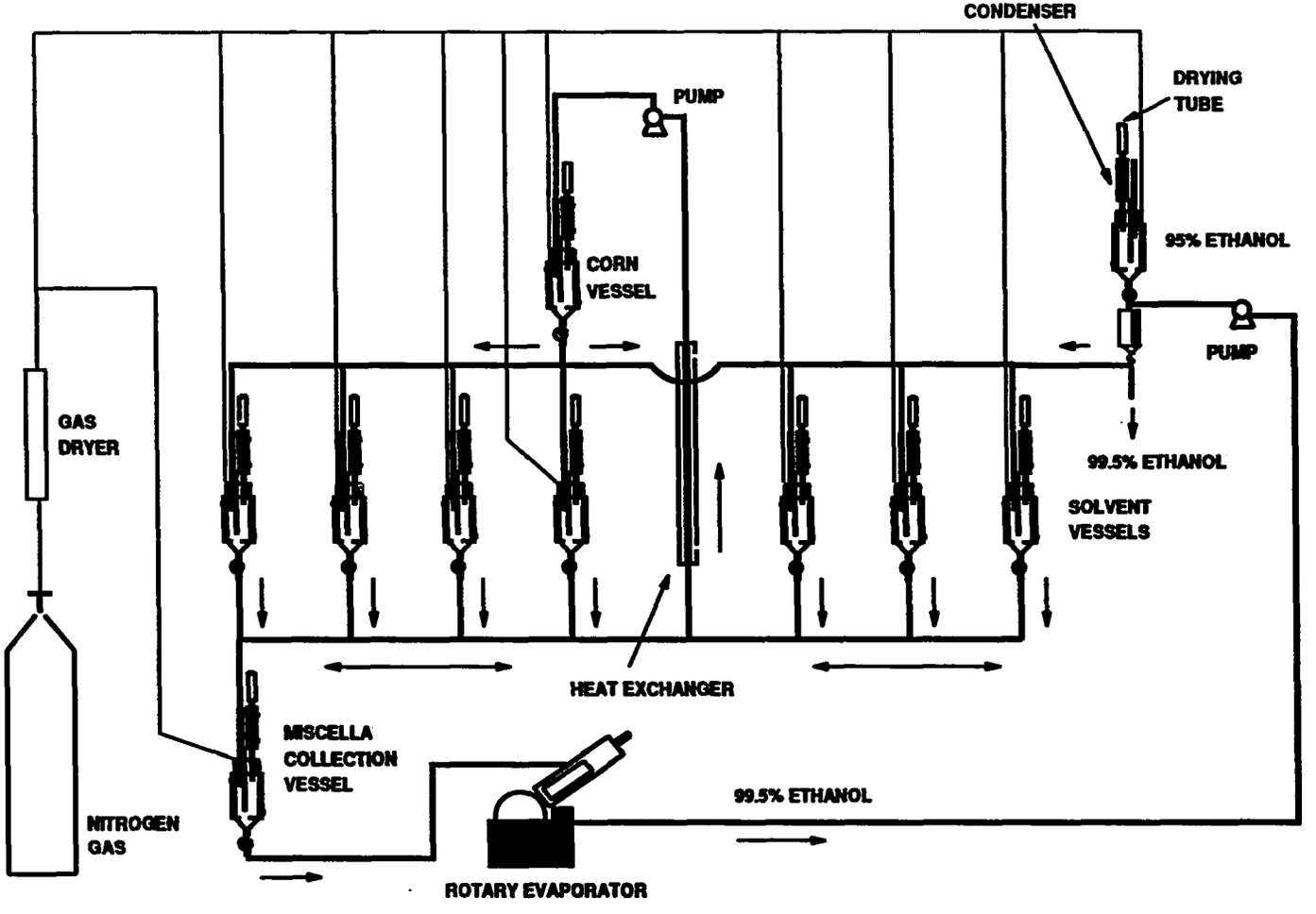


Figure 2. The countercurrent oil/moisture extraction system

The miscellas were pre-heated and maintained at 75^{o}C by circulating heated water through the jacketed glass vessels. Dried, flaked Pioneer 3732 corn was placed in the extraction vessel and was subjected to 7 extraction stages. In each stage, the solvent was circulated through the flakes for 10 min. Except for the first solvent vessel, the contents of each vessel were pumped into the previously emptied container after circulation thus advancing solvent flow. The flake bed was then allowed to drain by gravity for 5 min. After the first stage of extraction (oldest miscella), the miscella was drained into the recovery vessel and drawn by vacuum into the pre-weighed sample flask of the rotary evaporator (Figure 2). The alcohol was evaporated, condensed, and then pumped into a graduated separatory funnel where the volume was carefully measured. This dry alcohol was mixed with 95% ethanol in a specific ratio to produce a fresh preparation of 97.2% ethanol in solvent vessel number 7. The remaining dry ethanol in the graduated separatory funnel was drained into a pre-weighed screw-capped glass vial and stored in a desiccator for moisture analysis. The corn extraction vessel was disconnected from the system.

A small amount of the defatted flakes was placed in a screw-capped vial for moisture analysis while two portions were placed in separate pre-weighed petri dishes for volatiles, residual oil and crude protein determinations. The remaining flakes were weighed into six blendor cups in amounts equivalent to 25 g of dry corn (Figure 3). The mixture of 45% ethanol:55% 0.1 M NaOH (v/v) was added at a ratio of 1.5 ml/g dry corn. The contents of each cup were ground in a Waring Blendor at full speed for 1.5 min and then allowed to stand for 2 hr. After soaking, more ethanol:alkali mixture was added at a ratio of 13.5 ml/g dry corn and the mixture was blended for another 30 sec. The contents of the blendor cups were transferred to centrifuge bottles and residues in the cup were removed by repeated washings with the ethanol:alkali mixture. The bottles were capped tightly, placed in racks, and then immersed in a water bath maintained at 55^{o}C. The bottles were shaken for 2 hr at 130 rpm. After protein extraction, the bottles

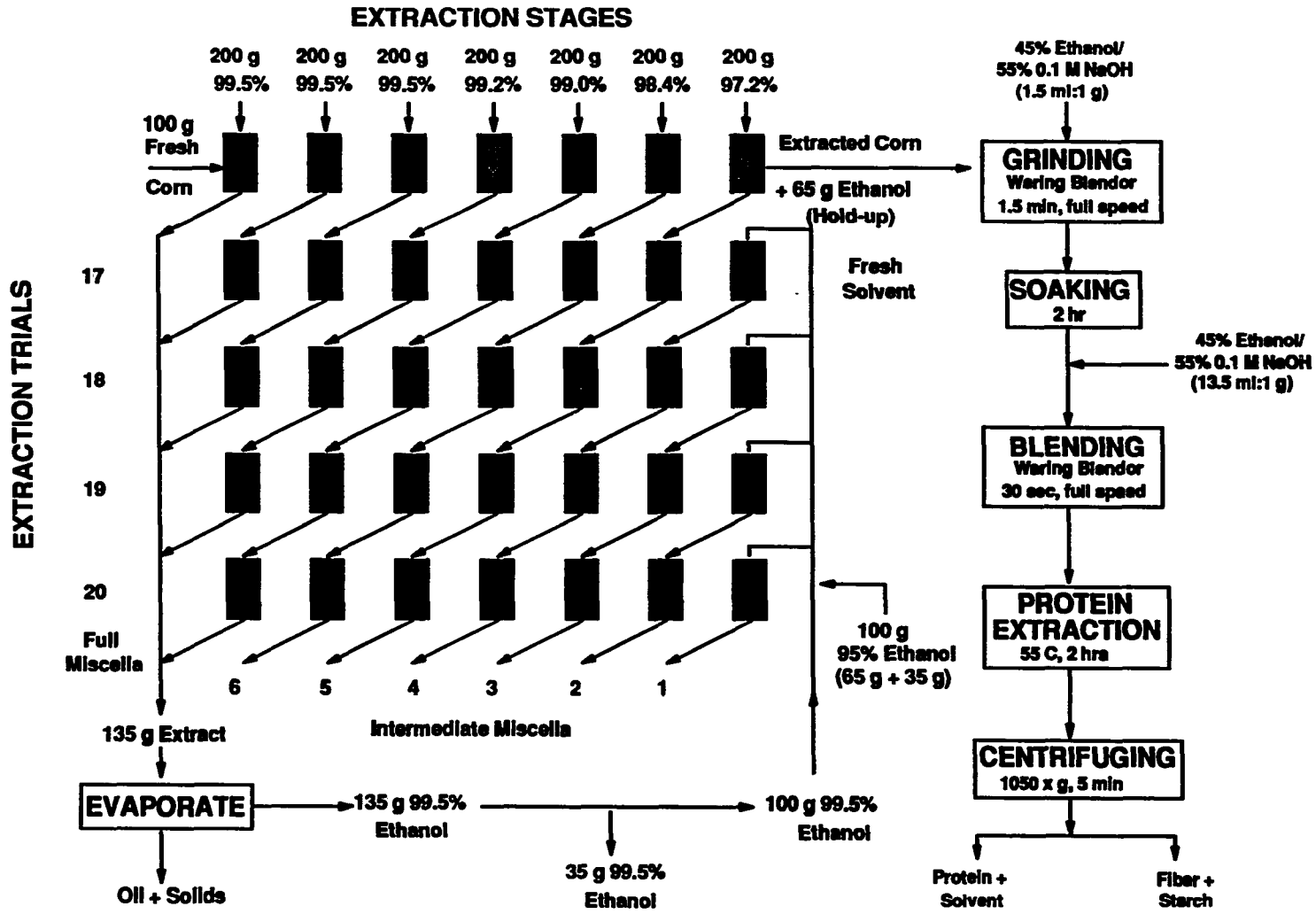


Figure 3. Schematic diagram of the sequential extraction process

were wiped dry and then centrifuged at 1050 x g for 5 min in a Sorvall Superspeed RC2-B centrifuge (Ivan Sorvall Inc., Newtown, CT). The supernatant was analyzed for crude protein content and the extraction efficiency was calculated. The residues (fiber + starch) were analyzed for moisture content and then dried in an oven at 105^{0}C prior to determining residual oil and crude protein contents. The sample flask from the rotary evaporator was also disconnected and set aside for oil recovery. The cleaned extraction vessel and a new sample flask were then replaced in the system for the succeeding extraction. The procedure was repeated four more times for Pioneer 3732 and five times for high-lysine corn.

Analyses of Samples

The Karl Fischer titration method (ASTM, 1975) was used to determine the moisture contents of the flaked corn before extraction, the start-up solvents, the defatted flakes immediately after extraction, the ethanol recovered from the full miscella, the residues extracted with the oil, the miscellas after the final extraction sequence for each variety, and the residue after protein extraction (fiber and starch).

The crude fat content of the flaked corn prior to extraction and the residual oil in the defatted corn, the residues extracted with the oil, and the fiber and starch were determined by AACC standard procedure 30-20 (AACC, 1983). The oil yield was determined for each run by extracting the oil and solids from the miscella with petroleum ether, filtering the washings into a pre-weighed flask and evaporating the solvent in a water bath. This procedure was also used to determine the amounts of oil in the miscellas after the final run.

AACC standard method 46-08 (AACC, 1983) was used to determine the crude protein contents of the flaked corn before extraction, the defatted flakes, the supernatant after protein extraction (protein extract), the residues extracted with the oil, and the fiber and starch.

Statistical Analyses

The data were analyzed using a Statistical Analysis System program (SAS, 1987). Significant differences among treatment means were identified by Least Significant Difference (LSD). Probability levels of $p \leq 0.05$ were considered significant. Evidence of significant differences is presented in the Appendix.

RESULTS AND DISCUSSION

Ethanol Dehydration

The moisture content of both corn varieties significantly increased after oil extraction (Table 1), indicating the adsorption of water from the solvent by the flaked corn bed. Although more water was adsorbed by the dent corn (Pioneer 3732), its water adsorption capacity of 19.9 g/kg corn was not significantly different from that of high-lysine corn which was 17.8 g water/kg corn (< 2% MC). The marked reduction in the moisture content of the ethanol recovered from the evaporation of the full miscella further verified the ethanol dehydration during the oil extraction process (Table 2). Both types of corn dried 95% ethanol to about 99% but Pioneer 3732 dehydrated the alcohol to a greater degree than did the high-lysine corn. The difference may have been due to the higher starting moisture content of the high-lysine corn (Table 1).

Table 1. Changes in the moisture contents of corn during oil extraction

	Pioneer 3732		High-lysine corn	
Run #	Initial MC[1] %	MC after oil removal, %	Initial MC %	MC after oil removal, %
1	0.81 ± 0.09	2.92 ± 0.12	1.48 ± 0.10	3.24 ± 0.00
2	0.86 ± 0.07	2.76 ± 0.02	1.46 ± 0.06	3.26 ± 0.02
3	0.92 ± 0.12	2.97 ± 0.01	1.48 ± 0.07	3.08 ± 0.03
4	1.26 ± 0.00	3.04 ± 0.01	1.42 ± 0.07	3.08 ± 0.02
5	0.98 ± 0.08	3.12 ± 0.02	1.11 ± 0.04	3.21 ± 0.01
Mean[2]	0.97 ± 0.18[a]	2.96 ± 0.14[b]	1.39 ± 0.16[c]	3.17 ± 0.09[d]

[1]MC denotes moisture content.

[2]Grand mean of five runs. Means with the same superscript are not significantly different at $p \leq 0.05$.

Table 2. Moisture content of ethanol recovered from the full miscella

Run no.	Ethanol moisture content, % (volume basis)	
	From Pioneer 3732 trials	From high-lysine trials
1	0.99 ± 0.01	1.28 ± 0.04
2	1.01 ± 0.03	1.22 ± 0.02
3	0.96 ± 0.01	1.29 ± 0.01
4	1.00 ± 0.01	1.26 ± 0.02
5	0.98 ± 0.03	1.28 ± 0.01
Grand Mean[1]	0.99 ± 0.02[a]	1.27 ± 0.03[b]

[1]Means with the same superscript are not significantly different at $p \leq 0.05$. Both values are significantly different from the moisture contents of 97.2% and 95.0% ethanol at $p < 0.01$.

The material balance on moisture content during the extraction of oil from Pioneer 3732 dent corn and high-lysine corn showed consistent data among the extraction trials and there was good agreement between the amount of water entering and leaving the system (Tables 3a and 3b).

Oil Extraction with Ethanol

The countercurrent system provided oil yields which were far superior to the estimated 72% recovery for conventional prepress hexane-extraction (Table 4). These results were also not significantly different from oil recoveries obtained from the earlier percolation extraction trials. Corn variety had no significant effect on the amount of crude oil extracted.

The profile of oil concentration in the miscellas for each extraction stage is given in Table 5. These values were determined after the fifth steady-state extraction trial for each type of corn. The highest oil concentrations were obtained in the first two stages

Table 3a. Water balance during oil/moisture extraction of Pioneer 3732 corn

Pioneer 3732				Moisture in		
Run #	Wt. corn g	MC[a] %	Total water in corn g	Water in 100 g 95% ethanol g	Water in 217 g 95% ethanol g	Total water g
1	221.67	0.81	1.80	6.15	13.34	15.14
2	219.85	0.86	1.89	6.15	13.34	15.23
3	221.77	0.92	2.04	6.15	13.34	15.38
4	221.83	1.26	2.80	6.15	13.34	16.14
5	225.34	0.98	2.21	6.15	13.34	15.55
Pioneer 3732				Moisture out		
Run #	Wt. marc g	MC %	Total water in marc g	Water in 100 g rec. ethanol[b] g	Water in total rec. ethanol g	Total water g
1	360.72	2.92	10.53	0.99	3.07	13.60
2	356.16	2.76	9.83	1.01	3.31	13.14
3	360.62	2.97	10.71	0.96	3.02	13.73
4	364.02	3.04	11.07	1.00	3.30	14.37
5	365.64	3.12	11.41	0.98	3.07	14.48

[a]MC denotes moisture content.

[b]Rec. ethanol denotes the alcohol recovered from evaporating the miscella.

of extraction. This was due to the fact that in countercurrent extraction, the fresh corn containing the maximum amount of oil for extraction comes in contact first with the oldest solvents (miscellas 1 and 2). Towards the last extraction stages, very little oil is available for recovery by the fresh solvents (miscellas 6 and 7). In addition, the miscellas from the first two extraction stages had the lowest moisture content and were closest to anhydrous levels (Table 6) where oil solubility is high.

Table 3b. Water balance during oil/moisture extraction of high-lysine corn

High-lysine corn				Moisture in		
Run #	Wt. corn g	MC[a] %	Total water in corn g	Water in 100 g 95% ethanol g	Water in 217 g 95% ethanol g	Total water g
1	207.06	1.48	3.06	6.15	13.34	16.40
2	203.93	1.46	2.98	6.15	13.34	16.32
3	207.77	1.48	3.07	6.15	13.34	16.41
4	205.14	1.42	2.91	6.15	13.34	16.25
5	206.18	1.11	2.29	6.15	13.34	15.63
High-lysine corn				**Moisture out**		
Run #	Wt. marc g	MC %	Total water in marc g	Water in 100 g rec. ethanol[b] g	Water in total rec. ethanol g	Total water g
1	331.05	3.24	10.73	1.28	3.90	14.63
2	323.00	3.26	10.53	1.22	3.66	14.19
3	333.28	3.08	10.26	1.29	4.06	14.32
4	331.90	3.08	10.22	1.26	4.03	14.25
5	335.50	3.21	10.77	1.28	3.84	14.61

[a]MC denotes moisture content.

[b]Rec. ethanol denotes the alcohol recovered from evaporating the miscella.

Protein Extraction

The crude protein contents of the dent corn and the high-lysine corn at various stages of the sequential extraction process are presented in Table 7. Ethanol has the capability of solubilizing and extracting small amounts of protein during oil extraction and a slight reduction in crude protein content was expected. However, the amount of protein which was co-extracted with the oil was negligible (Table 8). A significant

Table 4. Oil recovery from Pioneer 3732 and high-lysine corn using ethanol

Trial	Initial crude oil content, % db	Residual oil % db	Oil extraction efficiency, %
	Pioneer 3732		
1	3.53 ± 0.13	0.41 ± 0.04	88.4
2	4.20 ± 0.12	0.26 ± 0.02	93.8
3	3.67 ± 0.05	0.44 ± 0.03	88.0
4	4.18 ± 0.07	0.39 ± 0.04	90.7
5	3.57 ± 0.04	0.34 ± 0.04	90.5
Mean[1]	3.83 ± 0.33[a]	0.37 ± 0.07[b]	90.3 ± 2.3[c]
	High-lysine corn		
1	3.61 ± 0.10	0.46 ± 0.00	87.2
2	4.01 ± 0.13	0.13 ± 0.02	96.8
3	3.56 ± 0.01	0.22 ± 0.01	93.8
4	4.46 ± 0.04	0.19 ± 0.01	95.7
5	4.02 ± 0.25	0.21 ± 0.03	94.8
Mean	3.93 ± 0.36[a]	0.24 ± 0.13[b]	93.7 ± 3.7[c]

[1]Grand mean of five extraction trials. Means with the same superscript are not significantly different at $p < 0.05$.

reduction in protein yield was observed after extraction with the ethanol:NaOH mixture. More than 60% of the available protein was extracted by the mixture from both corn varieties. Similar values for protein extraction efficiency were obtained from calculations which used the protein content of the supernatant (ethanol:NaOH + protein) after centrifugation (Table 9). The type of corn did not significantly affect the protein yields. These protein yields were somewhat less than the protein recoveries obtained in Part III (72% and 70% for Pioneer 3732 dent corn and high-lysine corn, respectively) but they

Table 5. Oil concentration in the miscella at each extraction stage

Miscella No.	Oil content, g/100 g miscella	
	After Pioneer 3732 corn runs	After high-lysine corn runs
1 (Full)	3.34 ± 0.04	2.50 ± 0.06
2	2.23 ± 0.07	1.64 ± 0.10
3	0.76 ± 0.02	1.01 ± 0.03
4	0.49 ± 0.05	0.67 ± 0.08
5	0.39 ± 0.11	0.41 ± 0.13
6	0.44 ± 0.10	0.26 ± 0.08
7	0.08 ± 0.01	0.08 ± 0.00

Table 6. Moisture content profiles of miscellas at each extraction stage

Miscella No.	Moisture content, % volume basis	
	After Pioneer 3732 corn runs	After high-lysine corn runs
1 (Full)	1.30 ± 0.06	1.65 ± 0.01
2	1.38 ± 0.04	1.78 ± 0.01
3	1.70 ± 0.05	1.79 ± 0.03
4	1.78 ± 0.01	1.79 ± 0.00
5	1.88 ± 0.01	1.85 ± 0.01
6	1.94 ± 0.01	1.95 ± 0.00
7	2.04 ± 0.02	2.11 ± 0.02

were still significantly greater than the 48% expected protein recovery estimated from the protein solubility study in Part II. Random samples of the solubilized protein from Pioneer 3732 corn were dialyzed against water and then freeze-dried to recover the protein in solid form. The protein concentrate had an average crude protein content of 72.5% (db, Table 10). It was fibrous in appearance and had a bland flavor.

Table 7. Crude protein yields of dent corn and high-lysine corn during sequential extraction processing

Trial	Initial crude protein content g/100 g dry corn	CP[1] after oil extraction g/100 g dry residue	Residual CP in residue g/100 g dry residue	Protein recovery[2] %
	Pioneer 3732			
1	8.08 ± 0.00	8.32 ± 0.30	3.78 ± 0.11	54.6
2	9.40 ± 0.04	8.97 ± 0.24	3.20 ± 0.15	64.3
3	7.74 ± 0.04	7.63 ± 0.05	2.40 ± 0.03	68.5
4	9.43 ± 0.05	9.38 ± 0.02	2.39 ± 0.13	74.5
5	8.11 ± 0.05	8.06 ± 0.02	2.54 ± 0.12	68.5
Mean[3]	8.55 ± 0.80[a]	8.48 ± 0.69[a]	2.86 ± 0.61[b]	66.1 ± 7.4[c]
Trial	High-lysine corn			
1	8.93 ± 0.04	8.48 ± 0.02	3.22 ± 0.14	62.0
2	8.44 ± 0.02	8.24 ± 0.10	3.31 ± 0.07	59.7
3	8.14 ± 0.06	8.57 ± 0.39	3.28 ± 0.04	61.7
4	8.94 ± 0.13	9.12 ± 0.18	3.24 ± 0.02	64.5
5	9.25 ± 0.00	9.36 ± 0.13	3.18 ± 0.05	66.0
Mean	8.74 ± 0.44[a]	8.85 ± 0.37[a]	3.24 ± 0.05[b]	62.8 ± 2.5[c]

[1]CP denotes crude protein.

[2]Based on residual crude protein in fiber and starch. Means with the same superscript are not significantly different at $p \leq 0.05$.

[3]Grand mean of five extraction trials. Means with the same superscript are not significantly different at $p \leq 0.05$.

Table 8. Crude protein content of solids co-extracted with the oil

Trial	Pioneer 3732			High-lysine corn		
	Weight solids g	Protein in solids g/100 g dry solids	Protein extracted with oil g/100 g dry corn	Weight solids g	Protein in solids g/100 g dry solids	Protein extracted with oil g/100 g dry corn
1	4.52	21.73	0.44	6.45	29.28	0.92
2	6.86	29.10	0.91	6.43	30.57	0.97
3	6.25	32.28	0.91	6.33	29.30	0.90
4	6.82	30.08	0.92	6.29	26.88	0.83
5	6.44	30.37	0.86	6.24	25.11	0.77
Mean[1]	6.18[a] ± 0.96	28.71[b] ± 4.07	0.81[c] ± 0.18	6.35[a] ± 0.09	28.23[b] ± 2.20	0.88[c] ± 0.07

[1]Grand mean of five extraction trials. Means with the same superscript are not significantly different at $p \leq 0.05$.

Table 9. Amount of protein extracted from dent corn and high-lysine corn by 45% ethanol:55% 0.1 M NaOH

Trial	Pioneer 3732		High-lysine corn	
	Crude protein in extract g/100 g dry corn	Protein recovery[1] %	Crude protein in extract g/100 g dry corn	Protein recovery %
1	4.48 ± 0.45	53.8	5.24 ± 0.13	61.7
2	5.72 ± 0.45	63.9	5.38 ± 0.36	65.3
3	5.24 ± 0.04	68.7	5.24 ± 0.40	61.1
4	6.90 ± 0.09	73.6	5.96 ± 0.04	65.2
5	5.50 ± 0.22	68.2	6.18 ± 0.27	66.1
Mean[2]	5.57 ± 0.79[a]	65.4 ± 7.4[b]	5.60 ± 0.39[a]	63.9 ± 2.3[b]

[1]Based on protein content of the extract and protein content of corn after oil extraction given in Table 6.

2Grand mean of five extraction trials. Means with the same superscript are not significantly different at $p \leq 0.05$.

Table 10. Proximate analysis of freeze-dried protein concentrate from Pioneer 3732 corn

	Sample number				Mean Values
	1	2	3	4	
Moisture content, %	3.16	3.26	3.22	3.22	3.22
Crude protein, % db	77.22	71.20	73.40	68.20	72.50

SUMMARY AND CONCLUSIONS

The separate elements of the procedure worked well when they were integrated into a single process. Oil and protein yields and water adsorption capacity of the corn did not vary significantly from those obtained in the earlier separate phases of the research.

Ethanol extracted 90% of the oil in the corn, a recovery which is significantly greater than the 72% estimated for the conventional prepress hexane-extraction process. The moisture adsorption capacity of flaked dent corn was 20 g/kg corn at an initial moisture content of < 2%, while for flaked high-lysine corn, the adsorption capacity was 18 g/kg corn at an initial moisture content of < 2%. Both capacities were sufficient to dry 35 g of 95% ethanol/100 g corn at < 2% moisture content (2.5 gal/bu) to about 99% ethanol. The ethanol:NaOH mixture extracted over 60% of the available protein in the corn. The protein concentrate contained 72.5% crude protein (db). The type of corn had no significant effect on the oil and protein extraction efficiencies. The sequential extraction of dried, flaked whole corn appears technically viable and may have considerable economic potential in producing fuel ethanol from the fermentation of cornstarch.

REFERENCES

AACC. 1983. Approved methods. 8th ed. American Association of Cereal Chemists, Inc., St. Paul, MN.

ASTM. 1975. Standard test method for water using Karl Fischer reagent. ASTM designation: E 203-75 (Reapproved 1986). American Society for Testing and Materials, Philadelphia, PA.

ASTM. 1985. Standard test method for water in organic liquids by coulometric Karl Fischer titration. ASTM designation: E 1064-85. American Society for Testing and Materials, Philadelphia, PA.

Beckel, A. C., P. A. Belter, and A. K. Smith. 1948. The non-distillation alcohol extraction process for soybean oil. J. Am. Oil Chem. Soc. 25:10-11.

Chen, L. F., and D. F. Houston. 1970. Solubilization and recovery of protein from defatted rice bran. Cereal Chem. 47:72-79.

Chien, J. T., J. E. Hoff, and L. F. Chen. 1988. Simultaneous dehydration of 95% ethanol and extraction of crude oil from dried ground corn. Cereal Chem. 65(6):484-485.

Concon, J. M. 1973. Rapid and simple method for the quantitative extraction of corn endosperm proteins. Anal. Biochem. 55:563-572.

Harris, W. D., F. F. Bishop, C. M. Lyman, and R. Helpert. 1947. Isopropanol as a solvent for extraction of cottonseed oil. I. Preliminary investigations. J. Am. Oil Chem. Soc. 24:370-375.

Harris, W. D., J. W. Hayward, and R. A. Lamb. 1949. Isopropanol as a solvent for extraction for extraction of cottonseed oil. II. Separation of purified oil from miscella. J. Am. Oil Chem. Soc. 26:719-723.

Hassanen, N. Z., L. A. Johnson, J. T. Farnsworth, and E. W. Lusas. 1985. Sequential extraction process for extracting oil and aflatoxin from cottonseed. Abstract no. 81. J. Am. Oil Chem. Soc. 62(4):639.

Huster, H., F. Meuser, and C. Hoepke. 1983. Method of producing starch from grain or ground grain products by the wet process. United States Patent No. 4,416,701.

Karnofsky, G. B. 1981. Ethanol and isopropanol as a solvent for full-fat cottonseed extraction. Oil Mill Gaz. 85:34.

Ladisch, M. R. and G. T. Tsao. 1982. Vapor phase dehydration of aqueous alcohol mixtures. U.S. Patent No. 4,345,973.

Ladisch, M. R., M. Voloch, J. Hong, P. Blenkowski, and G. T. Tsao. 1984. Commeal adsorber for dehydrating ethanol vapors. Ind. Eng. Chem. Process Dev. 23:437-443.

Landry, J., J. W. Paulis, and D. A. Fey. 1983. Relationship between alcohol-soluble proteins extracted from maize endosperm by different methods. J. Agric. Food Chem. 31:1317-1322.

Lawhon, J. T. 1986. Process for recovery of protein from agricultural commodities prior to alcohol production. U.S. Patent No. 4,624,805.

Lusas, E. W., K. C. Rhee, and J. T. Lawhon. 1985. Recovery and utilization potential of proteins from corn and other cereals in alcohol production. International Conference on Ethanol from Biomass, Phillipsburg, Netherlands Antilles.

Meuser, F., and H. German. 1984. Use of high-pressure homogenization for the production of starch from maize. Starch 36:116-121.

Paulis, J. W. 1982. Recent developments in corn protein research. J. Agric. Food Chem. 30:14-20.

Rao, R. K., and L. K. Arnold. 1956a. Alcoholic extraction of vegetable oils. II. Solubilities of corn, linseed, and tung oils in aqueous ethanol. J. Am. Oil Chem. Soc. 33:82-84.

Rao, R. K., and L. K. Arnold. 1956b. Alcoholic extraction of vegetable oils. III. Solubilities of babassu, coconut, olive, palm, rapeseed, and sunflower seed oils in aqueous ethanol. J. Am. Oil Chem. Soc. 33:389-391.

Rao, R. K., and L. K. Arnold. 1957. Alcoholic extraction of vegetable oils. IV. Solubilities of vegetable oils in aqueous 2-propanol. J. Am. Oil Chem. Soc. 34:401-404.

Rao, R. K., M. G. Krishna, S. H. Zaheer, and L. K. Arnold. 1955. Alcoholic extraction of vegetable oils. I. Solubilities of cottonseed, peanut, sesame, and soybean oils in aqueous ethanol. J. Am. Oil Chem. Soc. 32:420-423.

Russell, M. H. 1980. Protein separation from corn endosperm by solvent extraction. Ph.D. Thesis. Purdue University, Lafayette, IN.

Russell, M. H., and G. T. Tsao. 1982. Protein separation from corn endosperm by solvent extraction. AIChE Symp. Ser. 78:83-89.

SAS. 1987. SAS/STAT guide for personal computers. Version 6 ed. SAS Inst. Inc., Cary, NC.

Swallen, L. C. 1941. Zein - a new industrial protein. Ind. Eng. Chem. 33(3):394-398.

Turner, J. E., J. A. Boundy, and R. A. Dimler. 1965. Zein: a heterogeneous protein containing disulfide-linked aggregates. Cereal Chem. 42:452-461.

GENERAL SUMMARY AND CONCLUSIONS

Ethanol, isopropanol, acetone, butanol, and the mixture of butanol:acetone:ethanol (6:3:1) extracted oil from dried, flaked whole corn in quantities nearly equal to or better than the 72% recovered by current technology employed in industry. Acetone removed other non-oil materials which were not identified. Anhydrous solvents and elevated extraction temperatures recovered more oil. Low temperature extraction appears feasible when using ethanol (40^{0}C), isopropanol (25^{0}C), and butanol: acetone:ethanol (25^{0}C).

Total crude protein content was significantly reduced in corn extracted with butanol, isopropanol, and ethanol, particularly when aqueous concentrations and high temperatures were used for extraction. Oil extraction with 67% butanol (75^{0}C) produced the greatest reduction in crude protein content of the corn.

Acetone, butanol, and butanol:acetone:ethanol (6:3:1) reduced the extractability of the different protein classes in the corn, particularly when higher temperatures (50-75^{0}C) were employed for oil extraction. Zein, the ethanol-soluble fraction, was the most severely affected by the extraction treatments. High temperature oil extraction was detrimental to the solubility of zein. The greatest decrease in the solubility of the proteins was observed in corn extracted with 67% butanol at 75^{0}C. Ethanol and isopropanol extracted oil with minimal denaturation of the corn proteins.

Medium-hard dent corn and soft dent corn showed maximum protein yields when extracted with 45% and 15% ethanol mixed with 0.100 M NaOH. High-lysine corn showed high protein yields when extracted with 0.100 M NaOH and with 45% ethanol:55% 0.125 M NaOH. The appearance of two sets of conditions which produced high protein yields suggests the strong probability of extracting two kinds of corn proteins and the possibility of maximizing protein recovery by using a two-stage extraction process. Protein extraction using 45% ethanol:55% 0.100 M NaOH at 50-60^{0}C was optimum for recovering protein from dried, flaked, undegermed corn. Neither

sonication at 10 KHz nor homogenization at 0.70 or 3.16 kg/mm^2 (1000 or 4500 psi) significantly increased the amount of protein extracted.

It is possible to simultaneously extract the oil from corn and dehydrate 95% ethanol to about 99% ethanol in a countercurrent extraction process using dried, flaked, undegermed corn at a 2:1 solvent:flake ratio.

The separate elements of sequential extraction processing worked well when they were integrated into a single countercurrent process. Ethanol (97.5%) extracted 90% of the oil in the corn, a recovery which was superior to the 72% estimated for the conventional prepress hexane-extraction process. The moisture adsorption capacities of 20 g/kg medium-hard dent corn (initial moisture < 2%) and 18 g/kg high-lysine corn (initial moisture < 2%) were sufficient to dry 35 g of 95% ethanol/100 g corn (2.5 gal/bu) to about 99% ethanol. The ethanol:NaOH mixture extracted over 60% of the available protein in the corn and the protein concentrate contained 72.5% crude protein (db).

The sequential extraction of flaked whole corn with ethanol appears technically feasible and may have considerable economic potential in producing fuel ethanol from cornstarch fermentation.

RECOMMENDATIONS

This study determined only the oil and protein yields from flaked, undegermed corn. The quality of the oil should also be evaluated. Attempts should be made to maximize protein yields using the two different alcohol concentrations. The use of membranes to facilitate protein recovery should be explored. Research on the composition of the extracted protein, as well as, on the functional properties and possible applications in food, are critical. These investigations, together with an economic evaluation of the complete process, would provide more information on the potential of the Sequential Extraction Process to produce quality oil, highly functional food-grade corn proteins, and anhydrous ethanol.

GENERAL REFERENCES

Beckel, A. C., P. A. Belter, and A. K. Smith. 1948a. Solvent effects on the products of soybean oil extraction. J. Am. Oil Chem. Soc. 25:7-9.

Beckel, A. C., P. A. Belter, and A. K. Smith. 1948b. The non-distillation alcohol extraction process for soybean oil. J. Am. Oil Chem. Soc. 25:10-11.

Chien, J. T., J. E. Hoff, and L. F. Chen. 1988. Simultaneous dehydration of 95% ethanol and extraction of crude oil from dried ground corn. Cereal Chem. 65(6):484-485.

Concon, J. M. 1973. Rapid and simple method for the quantitative extraction of corn endosperm proteins. Anal. Biochem. 55:563-572.

Harris, W. D., F. F. Bishop, C. M. Lyman, and R. Helpert. 1947. Isopropanol as a solvent for extraction of cottonseed oil. I. Preliminary investigations. J. Am. Oil Chem. Soc. 24:370-375.

Harris, W. D., J. W. Hayward, and R. A. Lamb. 1949. Isopropanol as a solvent for extraction of cottonseed oil. II. Separation of purified oil from the miscella. J. Am. Oil Chem. Soc. 26:719-723.

Hassanen, N. Z., L. A. Johnson, J. T. Farnsworth, and E. W. Lusas. 1985. Sequential extraction process for extracting oil and aflatoxin from cottonseed. Abstract no. 81. J. Am. Oil Chem. Soc. 62(4):639.

Hong, J., M. Voloch, M. R. Ladisch, and G. T. Tsao. 1982. Adsorption of ethanol-water mixtures by biomass materials. Biotechnology and bioengineering 24(3)725-730.

Hron, R. J., Sr., S. P. Koltun, and A. V. Graci. 1982. Biorenewable solvents for vegetable oil extraction. J. Am. Oil Chem. Soc. 59(9):674A-684A.

Huster, H., F. Meuser, and C. Hoepke. 1983. Method of producing starch from grain or ground grain products by the wet process. U.S. Patent No. 4,416,701.

Johnson, L. A., and E. W. Lusas. 1983. Comparison of alternative solvents for oils extraction. J. Am. Oil Chem. Soc. 60(2):229-241.

Karnofsky, G. B. 1981. Ethanol and isopropanol as solvents for full-fat cottonseed extraction. Oil Mill Gaz. 85:34.

Ladisch, M. R., and G. T. Tsao. 1982. Vapor phase dehydration of aqueous alcohol mixtures. U.S. Patent No. 4,345,973.

Ladisch, M. R., M. Voloch, J. Hong, P. Blenkowskl, and G. T. Tsao. 1984. Commeal adsorber for dehydrating ethanol vapors. Ind. Eng. Chem. Process Des. Dev. 23:437-443.

Landry, J., and T. Moureaux. 1970. Heterogeneite des glutelines du grain de mais: Extraction selective et composition en acides amines des trois fractions isolees. Bull. Soc. Chim. Biol. 52:1021-1037.

Lawhon, J. T. 1986. Process for recovery of protein from agricultural commodities prior to alcohol production. U.S. Patent No. 4,624,805.

Meuser, F., and H. German. 1984. Use of high-pressure homogenization for the production of starch from maize. Starch 36:116-121.

Osborne, T. B., and L. B. Mendel. 1914. Nutritive properties of the maize kernel. J. Biol. Chem. 18:1-16.

Paulis, J. W., and J. S. Wall. 1969. Albumins and globulins in extracts of corn grain parts. Cereal Chem. 46:265-273.

Rao, R. K., and L. K. Arnold. 1956a. Alcoholic extraction of vegetable oils. II. Solubilities of corn, linseed, and tung oils in aqueous ethanol. J. Am. Oil Chem. Soc. 33:82-84.

Rao, R. K., and L. K. Arnold. 1956b. Alcoholic extraction of vegetable oils. III. Solubilities of babassu, coconut, olive, palm, rapeseed, and sunflower seed oils in aqueous ethanol. J. Am. Oil Chem. Soc. 33:389-391.

Rao, R. K., M. G. Krishna, S. H. Zaheer, and L. K. Arnold. 1955. Alcoholic extraction of vegetable oils. I. Solubilities of cottonseed, peanut, sesame, and soybean oils in aqueous ethanol. J. Am. Oil Chem. Soc. 33:420-423.

Russell, M. H. 1980. Protein separation from corn endosperm by solvent extraction. Ph.D. Thesis. Purdue University, Lafayette, IN.

Vaccarino, G., and S. Vaccarino. 1961. A new industrial process for cottonseed. J. Am. Oil Chem. Soc. 38:143-147.

Watson, S. A., and P. E. Ramstad (eds.). 1987. Corn: Chemistry and technology. American Association of Cereal Chemists, Inc., St. Paul, MN.

Weber, E. J. 1978. The lipids of corn germ and endosperm. J. Am. Oil Chem. Soc. 56:637-641.

APPENDIX

Table A-1. Statistical analysis of oil recovery data based on residual oil content

Analysis of Variance Procedure

Dependent Variable: OILREC

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	19	2262.17	119.06	55.19	0.0001
Error	20	43.14	2.16		
Corrected Total	39	2305.31			

R-Square	C.V.	Root MSE	OILREC Mean
0.981285	1.64	1.49	89.23

Source	DF	Anova SS	Mean Square	F Value	Pr > F
TRT	19	2262.17	119.06	55.19	0.0001

T tests (LSD) for variable: OILREC

Alpha=0.05 df=20 MSE=2.157168

Critical Value of T= 2.09

Least Significant Difference= 3.0637

Table A-2. Statistical analysis of oil recovery data based on oil yield (including anhydrous acetone)

Analysis of Variance Procedure

Dependent Variable: OILREC

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	19	32674.32	1719.70	22.62	0.0001
Error	20	1520.61	76.03		
Corrected Total	39	34194.93			

R-Square	C.V.	Root MSE	OILREC Mean
0.955531	10.07	8.72	86.55

Source	DF	Anova SS	Mean Square	F Value	Pr > F
TRT	19	32674.32	1719.70	22.62	0.0001

T tests (LSD) for variable: OILREC

Alpha=0.05 df=20 MSE=76.03042

Critical Value of T= 2.09

Least Significant Difference= 18.189

Table A-3. Statistical analysis of oil recovery data based on oil yield (excluding anhydrous acetone)

Analysis of Variance Procedure

Dependent Variable: OILREC

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	17	4005.30	235.61	34.83	0.0001
Error	18	121.75	6.76		
Corrected Total	35	4127.05			

R-Square	C.V.	Root MSE	OILREC Mean
0.970499	3.32	2.60	78.27

Source	DF	Anova SS	Mean Square	F Value	Pr > F
TRT	17	4005.30	235.61	34.83	0.0001

T tests (LSD) for variable: OILREC

Alpha=0.05 df=18 MSE=6.764017

Critical Value of T= 2.10

Least Significant Difference= 5.464

Table A-4. Analysis of variance of protein recovery data

Dependent Variable: Recovery

Source	DF	Sum of Squares	Mean Square	F-value	Pr>F
Model	62	30360.00341	489.67747	44.86	0.0001
Error	126	1375.47300	10.91645		
Corrected Total	188	31735.47641			

R-Square	C.V.	Root MSE
0.956658	5.781578	3.304006

Source	F-Value	Pr > F
Variety	24.25	0.0001
Ethanol	301.02	0.0001
NaOH	22.59	0.0001
Variety*Ethanol	42.55	0.0001
Variety*NaOH	1.17	0.3257
Ethanol*NaOH	18.95	0.0001
Variety*Ethanol*NaOH	5.78	0.0001
Test of Hypotheses using the ANOVA MS for Variety*Ethanol*NaOH as an error term		
Variety	4.20	0.0273
Ethanol	52.12	0.0001
NaOH	3.91	0.0338
Variety*Ethanol	7.37	0.0001
Variety*NaOH	0.20	0.9341
Ethanol*NaOH	3.28	0.0064

Table A-5. Duncan's Multiple Range Test on treatment means for protein recovery data

Source	Mean	Duncan Grouping 0.05	0.01
Variety			
Hlys	58.41	A	A
Hard	58.25	A	A
Soft	54.78	B	B
Ethanol			
45	69.89	A	A
15	67.30	B	B
0	61.97	C	C
25	56.47	D	D
35	55.85	D	D
55	51.62	E	E
65	36.92	F	F
NaOH			
0.125	58.42	A	A
0.100	58.15	A	A
0.075	54.87	B	B

Table A-6. Yields of oil and ethanol-soluble solids in each extraction run

Run #	Wt. of corn g	Wt. oil g	Oil yield % db	Wt. solids g	Solids yield % db
1	231.8	6.50	2.85	2.66	1.17
2	229.4	8.17	3.62	2.33	1.03
3	229.7	9.16	4.05	3.82	1.69
4	233.2	9.21	4.01	3.60	1.57
5	231.7	10.91	4.79	4.55	2.00
6	230.7	9.75	4.29	4.67	2.05
7	230.7	10.50	4.62	5.59	2.46
8	230.5	10.17	4.46	6.47	2.84
9	231.0	10.59	4.66	6.48	2.85
10	233.1	11.09	4.83	7.87	3.42
11	227.2	11.03	4.92	8.40	3.75
12	233.0	9.79	4.28	8.64	3.78
13	230.2	12.18	5.38	9.03	3.99
14	231.7	10.10	4.44	8.82	3.88
15	228.9	9.39	4.15	10.48	4.63
16	231.0	11.05	4.83	10.01	4.38
17	227.8	10.06	4.47	13.48	5.99
18	231.4	9.94	4.35	10.81	4.72
19	227.9	9.96	4.42	10.50	4.66
20	231.3	11.17	4.88	11.90	5.20

Table A-7. Moisture content of flaked corn before and after oil extraction

Run number	Moisture content before extraction (% wt. basis)	Moisture content after extraction (% wt. basis)
1	1.66	NT[a]
2	1.65	NT
3	1.55	NT
4	1.45	NT
5	1.66	NT
6	1.35	NT
7	1.50	NT
8	1.16	NT
9	1.71	NT
10	1.38	2.74
11	1.40	2.78
12	1.83	2.97
13	1.70	3.54
14	1.31	3.54
15	1.18	3.61
16	1.12	3.54
17	1.17	3.84
18	1.11	3.68
19	1.12	3.68
20	1.04	3.68

[a]Not taken; equilibrium was still being established.

Table A-8. Moisture contents of start-up solvents, ethanol from miscella, ethanol from marc, and miscellas after the last extraction

Mean moisture content, % (volume basis)						
Extraction stage	Desired initial ethanol conc., %	Actual ethanol M.C.[a]	Miscella M.C.	Extraction run #	Ethanol from miscella	Ethanol from marc
1	99.5	0.52	1.55	10	1.46	5.03
2	99.5	0.52	1.52	11	1.34	4.77
3	99.5	0.52	1.52	12	1.22	5.70
4	99.2	0.75	1.58	13	1.22	5.11
5	99.0	0.95	1.61	14	1.16	5.09
6	98.4	1.65	1.66	15	1.11	4.74
7	97.2	2.80	2.76	16	1.13	4.55
	95.0	5.42		17	1.11	4.79
				18	1.10	4.93
				19	1.09	5.05
				20	1.12	5.02

[a]M.C. denotes moisture content.

Table A-9. Solvent hold-up in flaked corn

Run #	% Hold-up	Run #	% Hold-up
1	70.7	11	71.4
2	72.0	12	70.6
3	73.4	13	71.2
4	71.8	14	69.2
5	72.8	15	69.7
6	73.5	16	69.8
7	73.8	17	70.2
8	74.7	18	69.6
9	71.5	19	66.5
10	71.1	20	64.1

Table A-10. Statistical analysis of corn moisture content data after oil extraction

Analysis of Variance Procedure

Dependent Variable: MC

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	10	3.20840	0.3208	61.75	0.0001
Error	11	0.05715	0.0052		
Corrected Total	21	3.26555			

R-Square	C.V.	Root MSE	MC Mean
0.982499	2.104511	0.072080	3.4250

Source	DF	Anova SS	Mean Square	F Value	Pr > F
RUN	10	3.2084	0.32084	61.75	0.0001

T tests (LSD) for variable: MC

Alpha=0.05 df=11 MSE=0.005195
Critical Value of T= 2.20
Least Significant Difference= 0.1586

T Grouping	Mean	N	RUN
A	3.8450	2	17
B A	3.8100	2	18
B C	3.6750	2	19
C	3.6300	2	20
C	3.6100	2	15
C	3.5450	2	16
C	3.5400	2	13
C	3.5350	2	14
D	2.9750	2	12
E	2.7750	2	11
E	2.7350	2	10

Table A-11. Statistical analysis of moisture content data of ethanol from miscella

Analysis of Variance Procedure

Dependent Variable: MC

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	10	0.41455	0.0414	17.61	0.0001
Error	22	0.05180	0.0024		
Corrected Total	32	0.46635			

R-Square	C.V.	Root MSE	MC Mean
0.888925	4.084900	0.048524	1.1879

Source	DF	Anova SS	Mean Square	F Value	Pr > F
RUN	10	0.41455	0.041455	17.61	0.0001

T tests (LSD) for variable: MC

Alpha=0.05 df=22 MSE=0.002355
Critical Value of T= 2.07
Least Significant Difference= 0.0822

T Grouping	Mean	N	RUN
A	1.4633	3	10
B	1.3400	3	11
C	1.2200	3	12
C	1.2167	3	13
D C	1.1633	3	14
D	1.1300	3	16
D	1.1200	3	20
D	1.1100	3	15
D	1.1067	3	17
D	1.1033	3	18
D	1.0933	3	19

Table A-12. Statistical analysis of moisture content data of ethanol from marc

Analysis of Variance Procedure

Dependent Variable: MC

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	10	1.75713	0.1757	2.05	0.1280
Error	11	0.94460	0.0859		
Corrected Total	21	2.70173			

R-Square	C.V.	Root MSE	MC Mean
0.650372	5.882200	0.293040	4.98182

Source	DF	Anova SS	Mean Square	F Value	Pr > F
RUN	10	1.757127	0.17571273	2.05	0.1280

T tests (LSD) for variable: MC

Alpha=0.05 df=11 MSE=0.085873
Critical Value of T= 2.20
Least Significant Difference= 0.645

T Grouping	Mean	N	RUN
A	5.700	2	12
B A	5.115	2	13
B A	5.090	2	14
B	5.050	2	19
B	5.030	2	10
B	5.025	2	20
B	4.935	2	18
B	4.790	2	17
B	4.770	2	11
B	4.740	2	15
B	4.555	2	16

Table A-13. Statistical analysis of corn moisture content data before and after oil extraction

Analysis of Variance Procedure

Dependent Variable: MC

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	1	19.8147	19.8147	2525.24	0.0001
Error	10	0.0785	0.0078		
Corrected Total	11	19.8932			

R-Square	C.V.	Root MSE	MC Mean
0.996056	3.678121	0.088581	2.40833

Source	DF	Anova SS	Mean Square	F Value	Pr > F
RUN	1	19.81470	19.8147000	2525.24	0.0001

T tests (LSD) for variable: MC

Alpha=0.05 df=10 MSE=0.007847
Critical Value of T= 2.23
Least Significant Difference= 0.114

T Grouping	Mean	N	RUN
A	3.6933	6	After
B	1.1233	6	Before

Table A-14. Statistical analysis of ethanol moisture content data before and after oil extraction

Analysis of Variance Procedure

Dependent Variable: MC

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	1	9.9120	9.91203	847.53	0.0001
Error	12	0.1403	0.01170		
Corrected Total	13	10.0524			

R-Square	C.V.	Root MSE	MC Mean
0.986039	5.521602	0.108145	1.95857

Source	DF	Anova SS	Mean Square	F Value	Pr > F
RUN	1	9.912028	9.91202857	847.53	0.0001

T tests (LSD) for variable: MC

Alpha=0.05 df=12 MSE=0.011695
Critical Value of T= 2.18
Least Significant Difference= 0.1259

T Grouping	Mean	N	RUN
A	2.8000	7	Before
B	1.1171	7	After

Table A-15. Oil content of solids co-extracted with crude corn oil

Trial	Pioneer 3732			High-Lysine Corn		
	Yield g	Amt. oil in solids g/100 g dry solids	Amt. oil extracted g/100 g dry corn	Yield g	Amt. oil in solids g/100 g dry solids	Amt. oil extracted g/100 g dry corn
1	4.52	0.93 ± 0.32	0.02	6.43	4.02 ± 0.36	0.12
2	6.86	1.43 ± 0.20	0.04	6.40	3.08 ± 0.16	0.10
3	6.25	4.70 ± 0.06	0.13	6.33	0.97 ± 0.20	0.03
4	6.82	3.57 ± 0.22	0.11	6.29	3.11 ± 0.08	0.10
5	6.44	3.53 ± 0.08	0.10	6.24	4.97 ± 0.05	0.15

Table A-16. Statistical analysis of Pioneer 3732 and high-lysine corn moisture content data before and after oil extraction

Analysis of Variance Procedure

Dependent Variable: MC

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	3	4.50880	1.50293	72.62	0.0001
Error	16	0.33112	0.02070		
Corrected Total	19	4.83992			

R-Square	C.V.	Root MSE	MC Mean
0.931586	8.869146	0.143858	1.62200

Source	DF	Anova SS	Mean Square	F Value	Pr > F
TRT	3	4.50880	1.50293	72.62	0.0001

T tests (LSD) for variable: MC

Alpha=0.05 df=16 MSE=0.020695
Critical Value of T= 2.12
Least Significant Difference= 0.1929

Means with the same letter are not significantly different

T Grouping	Mean	N	TRT
A	2.1740	5	Hilysaft
B	1.9580	5	Pnraft
C	1.3900	5	Hilysbf
D	0.9660	5	Pnrbf

Table A-17. Statistical analysis of ethanol moisture content data before and after oil extraction of Pioneer 3732 and high-lysine corn

Analysis of Variance Procedure

Dependent Variable: MC

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	2	9.52297	4.76149	1078.07	0.0001
Error	12	0.05300	0.00442		
Corrected Total	14	9.57597			

R-Square	C.V.	Root MSE	MC Mean
0.994465	3.944876	0.066458	1.68467

Source	DF	Anova SS	Mean Square	F Value	Pr > F
TRT	2	9.52297	4.76149	1078.07	0.0001

T tests (LSD) for variable: MC

Alpha=0.05 df=12 MSE=0.004417
Critical Value of T= 2.18
Least Significant Difference= 0.0916

Means with the same letter are not significantly different

T Grouping	Mean	N	TRT
A	2.8000	5	Etohbf
B	1.2660	5	Hilysaf
C	0.9880	5	Pnraf

Table A-18. Statistical analysis of oil content data before and after extraction of Pioneer 3732 and high-lysine corn

Analysis of Variance Procedure

Dependent Variable: OIL

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	3	64.00458	21.33486	321.60	0.0001
Error	16	1.06144	0.06634		
Corrected Total	19	65.066020			

R-Square	C.V.	Root MSE	OILMEAN
0.983687	12.30605	0.257566	2.09300

Source	DF	Anova SS	Mean Square	F Value	Pr > F
TRT	3	64.00458	21.33486	321.60	0.0001

T tests (LSD) for variable: OIL

Alpha=0.05 df=16 MSE=0.06634
Critical Value of T= 2.12
Least Significant Difference= 0.3453

Means with the same letter are not significantly different

T Grouping	Mean	N	TRT
A	3.9320	5	Hilysbf
A	3.8300	5	Pnrbf
B	0.3680	5	Pnraf
B	0.2420	5	Hilysaf

Table A-19. Statistical analysis of protein content data before oil extraction, after oil extraction, and after protein extraction of Pioneer 3732 and high-lysine corn

Analysis of Variance Procedure

Dependent Variable: PROT

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	5	210.09611	42.01922	137.89	0.0001
Error	24	7.31328	0.30472		
Corrected Total	29	217.40939			

R-Square	C.V.	Root MSE	PROT MEAN
0.966362	8.130614	0.552014	6.78933

Source	DF	Anova SS	Mean Square	F Value	Pr > F
TRT	5	210.09611	42.01922	137.89	0.0001

T tests (LSD) for variable: PROT

Alpha=0.05 df=24 MSE=0.30472
Critical Value of T= 2.06
Least Significant Difference= 0.7206

Means with the same letter are not significantly different

T Grouping	Mean	N	TRT
A	8.854	5	Hlafoil
A	8.740	5	Hlbfoil
A	8.552	5	Pnrbfoil
A	8.482	5	Pnrafoil
B	3.246	5	Hlafprot
B	2.862	5	Pnafprot

Table A-20. Statistical analysis of Pioneer 3732 and high-lysine corn moisture adsorption capacity data

Analysis of Variance Procedure

Dependent Variable: ADSCAP

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	1	11.23600	11.23600	3.71	0.0903
Error	8	24.24400	3.03050		
Corrected Total	9	35.48000			

R-Square	C.V.	Root MSE	ADSCAP MEAN
0.316685	9.21076	1.740833	2.09300

Source	DF	Anova SS	Mean Square	F Value	Pr > F
VAR	1	11.23600	11.23600	3.71	0.0903

T tests (LSD) for variable: ADSCAP

Alpha=0.05 df=8 MSE=3.0305
Critical Value of T= 2.31
Least Significant Difference= 2.5389

Means with the same letter are not significantly different

T Grouping	Mean	N	TRT
A	19.960	5	Pnr
A	17.840	5	Hilys

Table A-21. Statistical analysis of oil extraction efficiency data from Pioneer 3732 and high-lysine corn

Analysis of Variance Procedure

Dependent Variable: OILEFF

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	1	28.93401	28.93401	2.98	0.1225
Error	8	77.64560	9.70570		
Corrected Total	9	106.57961			

R-Square	C.V.	Root MSE	OILEFF MEAN
0.271478	3.38752	3.115397	91.9670

Source	DF	Anova SS	Mean Square	F Value	Pr > F
VAR	1	28.93401	28.93401	2.98	0.1225

T tests (LSD) for variable: OILEFF

Alpha=0.05 df=8 MSE=9.7057
Critical Value of T= 2.31
Least Significant Difference= 4.5436

Means with the same letter are not significantly different

T Grouping	Mean	N	TRT
A	93.668	5	Hilys
A	90.266	5	Pnr

Table A-22. Statistical analysis of protein recovery data from Pioneer 3732 and high-lysine corn

Analysis of Variance Procedure

Dependent Variable: PROEFF

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	1	7.65625	7.65625	0.25	0.6286
Error	8	242.24044	30.28006		
Corrected Total	9	249.89669			

R-Square	C.V.	Root MSE	PROEFF MEAN
0.030638	8.495934	5.502732	64.7690

Source	DF	Anova SS	Mean Square	F Value	Pr > F
VAR	1	7.65625	7.65625	0.25	0.6286

T tests (LSD) for variable: PROEFF

Alpha=0.05 df=8 MSE=30.28005
Critical Value of T= 2.31
Least Significant Difference= 8.0254

Means with the same letter are not significantly different

T Grouping	Mean	N	TRT
A	65.644	5	Pnr
A	63.894	5	Hilys

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