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Influence of drying temperature on the solubility, the purity of isolates and the electrophoretic patterns of corn proteins

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ABSTRACT

A sequential extraction of proteins from whole corn kernels dried between 54 and 130 °C was performed in order to elucidate the effect of the drying temperature on the solubility, the purity and the electrophoretic patterns of the different classes of corn proteins. It was observed that albumin, globulin and zein solubilities dropped significantly when the drying temperature increased, while fractions solubilised as glutelin-G_2 and glutelin-G_3 increased until 110 °C before dropping slightly at 130 °C. The analysis of the solubility of different protein groups indicated that mechanisms other than the creation of new disulfide bonds between proteins occurred during the high temperature drying of corn. Except for glutelin-G_1 and zein isolates, which were highly pure, the purities of albumin, globulin, glutelin-G_2 and glutelin-G_3 isolates after dialysis were influenced by the drying temperature. Sodium dodecyl sulphate-polyacrylamide gel electrophoresis (SDS-PAGE) showed the disappearance of some water and salt-soluble polypeptides at high drying temperatures. The electrophoretic patterns of zein and glutelin-G_1 were not significantly modified, although the solubility of zein was affected by the drying temperature.

1. Introduction

Corn is an important source of protein. Globally, it contributes approximately 42 million tons of protein a year, which corresponds to approximately 15% of the world annual production of food-crop protein (Li & Vasal, 2004).

Drying with hot air is usually utilized to preserve corn by decreasing the water content. During this process, seeds can undergo different alterations. An important consideration when dealing with the heated air process is the drying temperature, which depends on the particular end use of grain and the proposed residence time of grain in dryers (Jayas & White, 2003). Although high drying temperatures have the advantage of reducing drying time and allow a rapid cadence of production during harvesting periods, Hall (1980) recommended 43, 54 and 82 °C as, respectively, the maximal allowed temperatures of heated air for seed, commercial grain and animal feed corn. However, nowadays it is common to find industrial dryers and pilot plant-size bed dryers operating with air heated above 120 °C (Pallai, Németh, & Mujumdar, 1987) in spite of consequences of high drying temperatures on properties of corn and it derivatives.

Indeed, above some critical temperatures, grains show damaged germinative capacity, poor salt-soluble protein contents (Lupano & Añon, 1986) and less millability (Lasseran, 1973). Numerous studies have shown that the drying of wet corn kernels above 70 °C can result in denaturation of proteins and endogenous proteolytic enzymes (Eckhoff, 2004). Researchers have assumed that the fractionation impairment of corn kernels, observed when drying temperature increased, is, at least partially, linked to changes induced in the chemical and physical properties of the proteins (Lasseran, 1973; Weller, Paulsen, & Steinberg, 1988; Wight, 1981).

Although the disadvantages of the thermal denaturation of corn protein in the post-harvesting process and the possible nutritional implication of high temperature treatment of corn have been cited in literature, few studies have characterised protein subgroups present in the whole corn kernel dried at high temperatures.

McGuire and Earle (1958) first studied the nature of changes that occurred in corn protein when kernels, at approximately 20% and 30% moisture content, were artificially dried at various temperatures between 48.9 °C and 93.3 °C to a final moisture content of 12%. They found that the yields of proteins extracted by

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water, saline and alkaline solutions decreased as the corn was successively dried at higher temperatures. The fractionation method used in the McGuire and Earle (1958) study does not allow separate extraction of the several protein groups present in corn and proposed linear relationships between the solubility of protein and the corn drying temperature have been subject to controversies.

Using a more suitable sequential solubilisation method, Wall, James, and Donaldson (1975) explored the nature of changes that occurred in corn proteins when high-moisture grains were dried with air temperatures ranging from 32 to 143 °C (with an effective maximum kernel temperature of 104 °C). They showed a substantial decrease of salt-soluble protein, a small decrease in alcohol-soluble protein and an increase of protein solubilised by dissociating agents such as sodium dodecyl sulphate (SDS) and 2-mercaptoethanol (2-ME).

Wight (1981) studied the solubility of water-extracted protein and residual protein extracted with a 0.1 M sodium phosphate buffer, pH 7.2, containing 1% of SDS and 1% v/v 2-ME in a borate buffer at pH 10. He did not present results on specific modifications that occurred in protein subgroups, such as globulin, zein and glutelins, during the corn drying.

Neumann, Wall, and Walker (1984) assumed that extensive heat treatment of native whole corn denatured proteins caused molecular aggregation through non-covalent hydrophobic interactions and (covalently) with new intermolecular disulfide cross-links that contributed to protein insolubility. The extraction schemes used in most of the previous studies did not allow complete sequential extraction of corn protein subgroups, which is necessary to explain the mechanism of protein denaturation during the hot air-drying of corn. Improved solubility studies and the characterisation of the prepared protein isolates are actually needed.

Indeed, the protein solubility profile is an excellent index of protein functionality, indicating potential applications (Hung & Zayas, 1992). The heat denaturation of protein can result in changes of the structural properties and can modify physicochemical properties, such as hydrophobicity which, in turn, are connected to many functional properties related to food protein (Townsend & Nakai, 1983).

Using a sequential extraction scheme based on the Osborne and Mendel (1914) method, with some modifications suggested by Landry and Moureaux (1970) and Paulis (1982), corn proteins were separated into five major groups and the influence of corn drying temperature on the solubilisation of each group was analyzed. Some of the major groups of protein extracted were separated into subgroups which were prepared by dialysis and freeze-drying before characterisation of their purity and analysis of their electrophoretic patterns.

2. Materials and methods

2.1. Fluidized-bed drying of corn kernel

The corn kernel used was a flint corn, of Baltimore variety, harvested at approximately 0.45 g of water/g of dry matter. The shelled corn was received in the laboratory immediately after harvesting and stored at −18 °C in sealed plastic bags until the drying was performed. Before drying, samples were equilibrated at ambient temperature for one night.

Drying was done in a laboratory fluidized-bed dryer in which air flow, previously heated, was pulsed. The dryings were carried out at temperatures between 54 and 130 °C in triplicate. Undried corn was used as control. For each air-drying temperature, the processing time was chosen in order to obtain final moisture contents between 0.12 and 0.15 g of water/g of dry matter. The final kernel temperatures were close to those of air-drying. After drying, the corn kernels were equilibrated at ambient temperature and conserved in sealed bags at 10 °C.

2.2. Sample preparation

Before protein extraction, the air-dried corn kernels and the freeze-dried corn used as control were first ground in a Falling Number laboratory mill (Type 3100, Huddinge, Sweden). The median diameters of meals obtained were measured using a laser granulometer (Malvern, Mastersizer 2000, UK) and were less than 0.2 mm for all samples.

Twenty grams of ground meals were twice defatted by stirring with 180 ml of 80% hexane + 20% diethyl ether (v/v) for 1 h at ambient temperature and decanted for 30 min. Supernatant was carefully siphoned and filtered on a Buchner funnel. Solid residues were air-dried at ambient temperature before the subsequent protein extractions.

2.3. General scheme of sequential extraction of protein

The sequential extraction procedure of corn protein was developed by combining the methods described by Landry and Moureaux (1970) and Paulis (1982). For comparison purposes, Table 1 summarizes the solvent used, the stirring time, the temperature applied for each step of sequential extraction as performed in the present study and that described in the literature. The general extraction scheme and the fractionation of specific groups of protein, as applied in the present study, are presented in Fig. 1.

Centrifugation was performed with a Beckman J2-21 centrifuge and a JA14 rotor, (Beckman LTD, UK) at 10,000*g* for 30 min. The supernatants from centrifugation were filtered using a Whatman®filtre 2V. The nitrogen contents of extracts from the general scheme of fractionation were determined before the fractionation of specific subgroups of protein.

2.4. Extraction of specific subgroups of protein and preparation of isolates

Subsequently to the general scheme of extraction (Fig. 1), a direct sequential extraction of albumin and globulin from the defatted corn meal was performed. Total water-soluble nitrogen-containing substances were first extracted by stirring 20 g of defatted corn meal with 200 ml of distilled water at 4 °C for 90 min before centrifugation. This operation was repeated twice with 150 ml of water and all the supernatants were combined as water-soluble nitrogen-containing substances.

Albumin isolated in water-soluble extract was separated from non-protein nitrogen-containing materials by precipitation of an aliquot of water extract after adding trichloroacetic acid to a final concentration of 10% (w/v) (Landry, Delhaye, & Di Goiai, 1999). The mixture was kept for one night at 4 °C before centrifuging at 4 °C. Nitrogen measured in the supernatant was considered as non-albumin nitrogen-containing substances and the difference from the total nitrogen content of the water extract was considered as albumin material.

The meal residues from water-soluble extraction were stirred for 90 min at 4 °C with 180 ml of 0.5 M of NaCl and then centrifuged at 10,000*g* for 30 min. Residues were extracted twice more under similar conditions. Globulin was separated from non-globulin nitrogen-containing substances by precipitation with trichloroacetic acid, as described previously.

After albumin, globulin and zein were removed, three groups of glutelins, named glutelin-G_1 (ASG), glutelin-G_2 and glutelin-G_3,

Table 1
Protein extraction procedure used in the present study compared to sequential extraction described in literature

	Present study	Moueium et al. (1996)	Landry and Moureaux (1981)	Landry and Moureaux (1980)	Paulis and Wall (1977)	Sodek and Wilson (1971)	Landry and Moureaux (1970)	Paulis et al. (1969)
Free amino acids						Water, 0 °C; 30, 30, 30		
Albumins and globulin	0.5 M NaCl, 4 °C, 10:1, 10:1, 5:1 v/w: 90, 90, 90	0.5 M NaCl, 4 °C, 10:1 v/w: 60, 30, 30; 30,000*g*	0.5 M NaCl, 4 °C, 10:1 v/w: 60, 30, 30	0.5 M NaCl, 5 °C, 10:1 v/w: 60, 30,000*g*	0.5 M NaCl, 4 °C, 5 v/w: 60, 60	0.5 M NaCl, 0 °C: 30, 30, 30	0.5 M NaCl, 4 °C, 10:1 v/w: 60, 30, 30; 30,000*g* 15 min	0.5 M NaCl, 5 °C, 5:1 v/w: 60, 60, 60
Zein	70% Ethanol + 0.5% sodium acetate, RT, 10:1, 10:1, 5:1 v/w: 90, 90, 90 min	60% Ethanol, 20 °C, 10:1 v/w, 30% and 55% isopropanol, 20 °C, 10:1 v/w: 60, 30, 15	55% Isopropanol (20 °C) 10:1 v/w: 60, 30, 15,	55% Isopropanol 10:1 v/w: 60, 30	70% Ethanol + 0.5% sodium acetate, RT, 5 v/w: 60, 60, 60	55% 2-Propanol, 20 °C, 3, 120, 60	55% Isopropanol, 20 °C, 30, 30, 30 10 v/w: 30,000*g*	70% Ethanol + 0.5% sodium acetate, RT, 5 v/w: 180, 180, 180
Zein-like glutelins (zein 2)	70% Ethanol = 0.5% sodium acetate + 0.1 M 2-ME, RT, 10:1, 10:1, 5:1 v/w: 90, 90, 90	60% Ethanol + 0.6% 2-ME, 10 :1 v/w, 30 min + 55% isopropanol, 20 °C 10:1 v/w + 0.6% 2 ME, RT, 10 v/w: 30,	55% Isopropanol 10:1 v/w + 0.6% 2-ME, RT, 10 v/w: 30, 30	55% Isopropanol 10:1 v/w + 0.6% 2-ME, RT, 10 v/w: 30, 30	70% Ethanol 0.5% sodium acetate + 2% 2-ME, RT, 10 v/W: 30, 30	55% 2-Propanol, 0.6% 2-ME, 20 v/w: 7 30, 60, 30	55% Isopropanol 10:1 v/w + 0.6% 2-ME, 20 °C, 10 v/w: 30, 30	
Glutelin-G_2	pH 10 Borate Buffer + 0.6% 2-ME, 20 °C, 10:1, 10:1, 5:1 v/w: 90, 90	pH 10 borate buffer + 0.6% 2-ME, 20 °C + 0.5 M NaCl, 10:1 v/w: 60, 30, 15	pH 10 borate buffer + 0.6% 2-ME, 0.5 M NaCl 60, 30, 15	pH 10 borate buffer + 0.6% 2-ME, 20 °C, 60, 30, 15			pH 10 borate buffer + 0,6% 2-ME, 20 °C, 60, 30, 15	
Glutelin-G_3	pH 10 Borate Buffer + 0.5% SDS + 0.6% 2-ME + 0.5% SDS, 10:1, 10:1, 5:1 v/w 90, 90, 90	pH 10 borate buffer + 0.5% SDS + 0.6% 2-ME + 0.5% SDS, 60, 30, 15	pH 10 borate buffer + 0.5% SDS + 0.6% 2-ME + 0.5 SDS, 60, 30, 15	pH 10 borate buffer + 0.5% SDS + 0.6% 2-ME + 0.5% SDS, 60, 30, 15			pH 10 borate buffer + 0.5% SDS + 0.6% 2-ME + 0.5% SDS, 60, 30, 15	
Glutelins $G_2 + G_3$						0.2% NaOH, 20 °C: 30, 120, 120		0.2% NaOH, 5:1 v/w, 3 °C: 120, 120, 120

RT: room temperature.

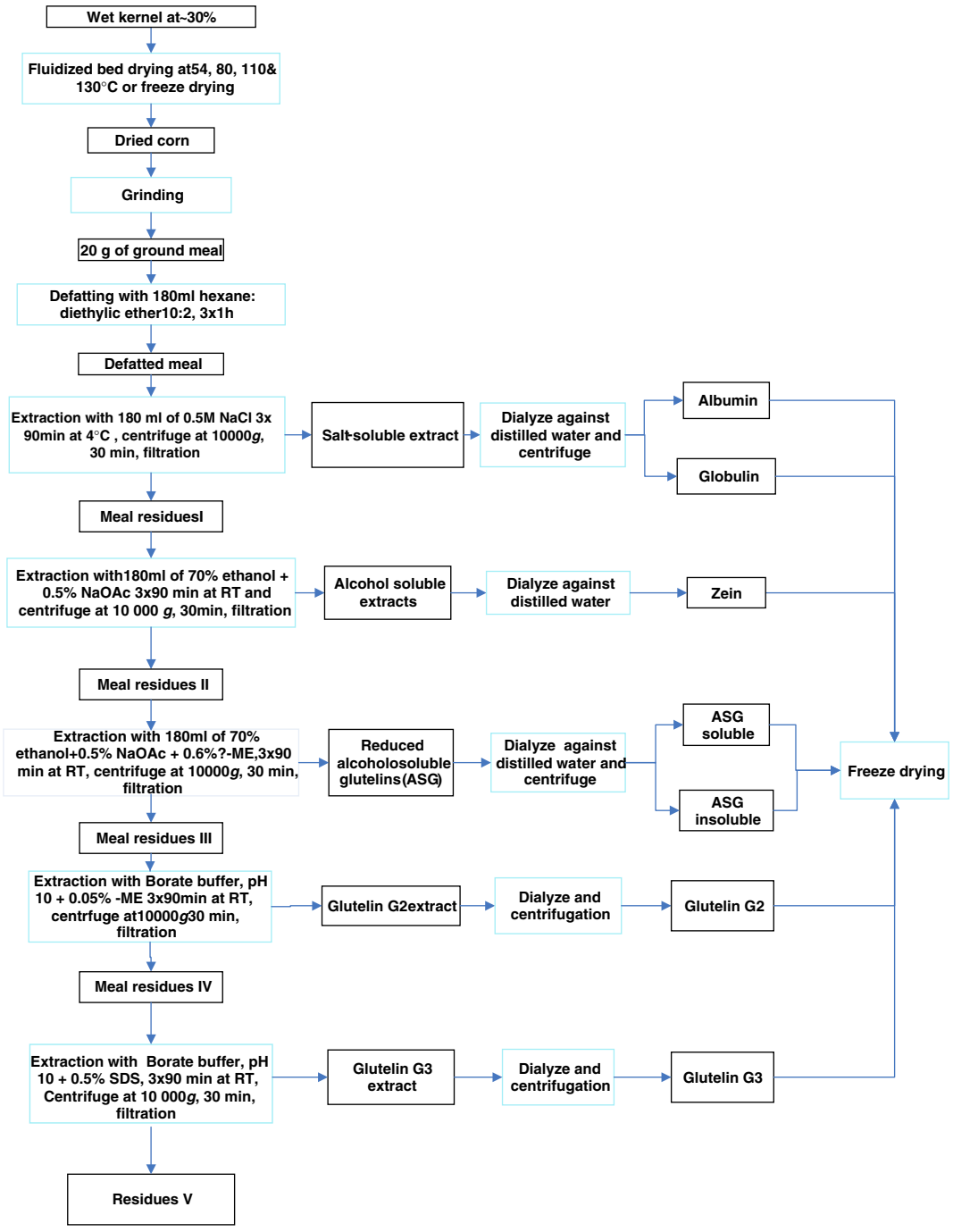

Fig. 1. General scheme of sequential extraction of corn protein and isolation of relative purified protein subgroups.

were successively extracted from the meal residues. The ASG extract, also zein-like glutelin (Landry & Moureaux, 1970), was then dialyzed against water at 4 °C and the fractionated residue after centrifugation was named water-insoluble ASG and water-soluble ASG, according to Paulis and Wall (1977).

Extracted protein subgroups were dialyzed against distilled water at 4 °C in a spectrapor molecular-porous membrane tubing (Spectrum Laboratories, Inc., Canada) with a molecular cutoff of 6000–8000 Da, according to the method described by Paulis and Wall (1977). The distilled water for dialysis was continuously stirred and usually renewed until the dialysis buffer was stabilized at a conductivity value less than 2 μS/cm. Dialyzed material were then freeze-dried to obtain the protein isolates.

2.5. Analytical methods

The moisture contents of the corn meal and the meal residues were determined by measuring the weight loss of a 5.0 g sample after 165 min at 130 °C (ISO 712:1998).

Nitrogen contents of extracts and isolates were determined by the Kjeldahl method, with a 2020 Tecator Digester (Tecator, Sweden) and a 2100 Kjeltec distiller (Tecator, Sweden). The percentage of protein was calculated using the general factor 6.25.

The purity of each isolate was calculated as the ratio of the protein content to the mass of the isolate on a dry basis. All reagents used were of analytical grade.

2.6. Electrophoretic characterisation of isolates

Horizontal sodium dodecyl-sulfate polyacrylamide gel electrophoresis (SDS-PAGE) of protein isolates was performed using a 8–18 gradient SDS-PAGE gel, together with ExcelGel D buffer strips ready to use (GE Healthcare Bio-sciences, Sweden).

For the development of electrophoretic patterns, 4 mg of each isolate were denatured and reduced by heating samples at 95 °C for 5 min in 1 ml of a buffer containing 1% of SDS, 0.25% of 2-ME, 0.01% of bromophenol blue, 20% of pH 7.5 tris buffer adjusted by acetic acid. For the non-reducing treatments, proteins were treated in SDS-containing sample buffer without 2-ME. This leaves disulfide bridges between and within the chains of polypeptides intact.

Denatured protein solutions were loaded into polyacrylamide gel lanes and migrated at 600 V using a LKB 2117 multiphor II electrophoresis unit and a LKB Bromma 2197 power supply (LKB, Sweden). Runs were stopped when the running front reached the end of the gel, and then stained using Coomassie blue.

A set of commercial protein markers with phosphorylase b (97 kDa), bovine serum albumin (66 kDa), ovalbumin (45 kDa), carbonic anhydrase (30 kDa), soybean trypsin inhibitor (20.1 kDa) and a-lactalbumin (14.4 kDa) (LMW, GE Healthcare, Sweden), was used to determine the molecular weights (Mw) of polypeptide bands of the isolates.

2.7. Statistical analysis

Statistical analyses were performed using Minitab software (version 14, MINITAB Inc., State College, PA) for the correlation analysis and one way ANOVA. Experiments were performed, at least in triplicate, and results are presented as means ± standard deviation.

3. Results and discussion

3.1. Performance of the protein fractionation scheme

Corn proteins were separated into five major groups. Successive extractions resulted in the release of salt-soluble proteins, zein and three glutelin groups, denoted as glutelin-G_1, glutelin-G_2 and glutelin-G_3. Table 2 presents the balance of extracted proteins, as observed using the sequential extraction scheme developed in the present study, and those presented in previous studies. Non-protein-nitrogen was converted into protein equivalent by using a factor 6.25.

For the corn kernel variety and the extraction scheme used in the present study, zein was the most abundant protein group extracted from the whole corn kernel, followed by glutelin-G_1 and glutelin-G_3.

The amount of zein recovered was in the range of results observed by Moueium, El Tinay, and Abdalla (1996), Paulis, James, and Wall (1969) and Sodek and Wilson (1971). Differences observed between the present study and previous studies are possibly due to the corn cultivar used.

As was observed by Landry and Moureaux (1981), the use of sodium acetate in alcohol containing a reducing agent, such as 2-ME, increased the nitrogen-containing substances extracted as glutelin-G_1 (zein-like glutelin) and significantly decreased nitrogen-containing substances extracted as glutelin-G_2. This explains differences observed in amounts of glutelin-G_1 and glutelin-G_2 comparatively to results reported by Landry and Moureaux (1970, 1980, 1981), in which sodium acetate was not used during alcohol extraction.

The amount of total salt-soluble nitrogen-containing substances was 18.2%. Paulis and Wall (1969) obtained 17% under similar conditions. The salt-soluble proteins obtained, after the removal of non-protein nitrogen, represents 10.5%. This fraction contains albumins and globulins which were subsequently resolved.

The sum of the all glutelin fractions ($G_1 + G_2 + G_3$) could be considered as the most important class of protein present in the whole flint corn variety used in the present study. However, the sum of zein-like protein (zein + zein-like glutelin) was the most important protein group recovered.

3.2. Influence of air-drying temperature on sequential protein extraction of corn kernel

The amounts of proteins sequentially extracted from corn grain dried at different temperatures are presented in Table 3. Except for the amount of total salt-soluble proteins recovered, no significant differences were observed between air-dried corn at 54 °C and freeze-dried corn.

Above 54 °C, salt-soluble proteins and zein dropped continuously with increase of the drying temperature. This result is consistent with those of Wall et al. (1975), McGuire and Earle (1958) and Lasseran (1973).

Table 2
Amounts of proteins (%) sequentially extracted from the whole native corn kernel

	Present study[a]	Moueium et al. (1996)[b]	Landry and Moureaux (1981)[c]	Landry and Moureaux (1980)[d]	Paulis and Wall (1977)[e]	Sodek and Wilson (1971)	Landry and Moureaux (1970)[f]	Paulis et al. (1969)[g]
Non-protein nitrogen equival.	7.7			6.8		4.4		
Total salino-soluble protein	10.5	19.5–26.2	19.0[h]	15.6	19[h]	2.4	19	7.8–20.2
Zein	33.5	18.3–35.4	38.0	39.4	41.1	36.9	38	45.8–24.4
Glutelin-G_1 (ASG)	21.8	20.9–35.3	11.5	9.4	12.6	18.5	11.5	na
Glutelin-G_2	1.5	0.91–1.80	10.0	9.3			10	na
Glutelin-G_3	18.1	16.3–23.8	18.0	13.4		22.9	18	37.5–46.2
Residues (insolubles)	6.7	1.4–2.4	3.5				3.5	
Total protein recovered	99.8	95.6–103.0	100			85.1		
Zein + zein-like glutelin	55.3	48.8–60.8	56.2	48.8	53.7	55.4	49.5	na
Glutelin G_1 + G2 + G_3	41.4	40.5–51.4	39.5	32.1		41.4	39.5	37.5–46.2

[a] Fraction from freeze-dried corn (native corn).
[b] Results mentioned are the range of ten cultivars.
[c] Results mentioned are those of the crop I_1 of INRA 260 cultivar following the scheme D_0.
[d] Results mentioned are those of whole grain of INRA 260 cultivar (I_1).
[e] Results mentioned are those of normal corn.
[f] Results presented are those of sequence D of Landry and Moureaux (1970).
[g] Results mentioned are those of normal and high-lysine crops (normal-high lysine).
[h] Fractions containing non-protein nitrogen equivalent.

Table 3
Influence of the drying temperature on the solubilisation of different classes of corn protein (% of total N)

Fraction yield	Air temperature drying (°C)				
	Freeze-dried	54	80	110	130
Non-protein nitrogen (protein equivalent)	7.7 ± 0.1	8.3 ± 0.0	6.6 ± 0.4	5.5 ± 0.1	4.9 ± 0.2
Total salt-soluble proteins	10.5 ± 0.2	9.0 ± 0.3	6.5 ± 1.1	3.4 ± 0.3	2.5 ± 0.4
Albumin	4.1 ± 0.0	3.6 ± 0.5	1.93 ± 0.3	1.6 ± 0.1	1.3 ± 0.2
Globulin	4.2 ± 0.1	3.9 ± 0.3	3.1 ± 0.1	1.7 ± 0.3	0.9 ± 0.2
Zein	33.5 ± 0.7	33.0 ± 1.8	25.9 ± 1.3	21.0 ± 2.0	15.7 ± 1.2
Glutelin-G_1	21.8 ± 1.8	20.9 ± 3.1	21.4 ± 3.3	20.3 ± 2.0	19.1 ± 2.8
Glutelin-G_2	1.5 ± 0.3	1.6 ± 0.5	2.0 ± 0.4	3.2 ± 0.7	2.8 ± 0.7
Glutelin-G_3	18.1 ± 1.2	19.9 ± 0.9	28.3 ± 2.9	34.6 ± 2.0	26.6 ± 2.0
Unfractionated proteins	6.9 ± 0.6	7.2 ± 0.5	8.8 ± 1.3	10.4 ± 2.0	24.0 ± 3.9
Total nitrogen yield	100.5 ± 4.7	99.9 ± 7.1	99.4 ± 9.9	98.0 ± 9.1	95.4 ± 10.1

As observed by Wall et al. (1975), the decrease of salt-soluble protein with the drying temperature does not follow a simple linear relationship. At higher drying temperatures, the decreasing rate of salt-soluble proteins steepens before dropping below an inflection point. A sigmoidal regression would correctly fit the relationship between the amounts of total salt-soluble protein recovered and the drying temperatures.

Albumin appeared as the most readily heat-denatured protein. The denaturation of albumins, which are mainly enzymatic proteins, may explain the loss of corn grain viability, even at moderate drying temperatures. Indeed, the germination phenomenon closely depends on the activation of seed enzymes (Bewley & Black, 1978), which may be denatured even at a moderate temperature.

The zein yield dropped by 18% when the drying temperature reached 130 °C. The loss of zein can not be explained only by new disulfide bonds created when sulfhydryl groups are oxidized or when intramolecular disulfide bonds are converted into intermolecular bonds. The use of a reducing agent, such as 2-ME, did not increase the yield of reduced alcohol-soluble protein yield (glutelin-G_1) from the corn dried at temperatures which induce significant zein insolubilization. Other modifications of zein structure, e.g. hydrophobic interactions, folding of chains or new covalent bonds with other material, certainly occurred at a high drying temperature.

A slight increase of recovered glutelin-G_2, which has a similar amino acid composition to salt-soluble proteins (Landry & Moureaux, 1981), in which disulfide bonds are broken by 2-ME, can not explain the decreasing rate of salt-soluble proteins, when corn kernels were dried above 54 °C. This confirms that mechanisms other than disulfide bonds, such as non-covalent interpolypeptide bonds, are associated with the denaturation of salt-soluble proteins and zein.

The amounts of the fraction recovered as glutelin-G_3 increased significantly above 54 °C, before decreasing beyond 110 °C. At 130 °C most of the proteins became insoluble, even in pH 10 borate buffers containing unfolding and reducing agents, such as SDS and 2-ME. The increase of insoluble nitrogen at 130 °C denoted a deep denaturation of corn proteins. The drying of corn at 130 °C also results in a marked browning of grain, indicative of extensive Maillard reactions between some protein residues and other materials present in corn grain, as was predicted by Wall et al. (1975).

Table 4 summarizes the correlation coefficients between the percentages of total protein recovered in different groups of protein extracted.

Except for glutelin-G_1, other protein groups were significantly correlated with the drying temperature. The decreasing amounts of total salt-soluble proteins and globulin extracted were highly correlated with the increase of glutelin-G_3 and glutelin-G_2. Probably most of denatured globulin was recovered as glutelin-G_2 and glutelin-G_3.

The solubility of globulin was highly correlated with that of zein ($r = 0.990$). The decrease in solubility of these two groups of proteins appeared to be similar to the increase of drying temperature.

The significant amounts of nitrogen contained in the insoluble fraction indicated that the dispersibility of protein from the whole dried corn was significantly affected at 130 °C. This may have consequences on the functional properties of cornprotein-based products. Townsend and Nakai (1983) observed that the dispersibilities of food proteins were highly correlated with protein hydrophobicity which, in turn, is relatable to many functional properties linked to food proteins, such as the foaming capacity.

In contrast to the conclusion of Wall et al. (1975) about dialyzable protein, non-protein nitrogen soluble in trichloroacetic acid increased slightly when corn was dried at 54 °C and then decreased continuously with increase of drying temperature ($r = -0.974$).

3.3. Influence of air-drying temperature on the purity of dialyzed protein isolates

The drying temperature influenced the composition of some protein isolates as shown in the Table 5.

The purity of albumin and globulin isolates decreased continuously whereas the purity of glutelin-G_2 and G_3 isolates increased with increase of drying temperature (Table 3). Globulin isolate had a higher nitrogen content than had albumin. Likewise, the glutelin-G_3 isolate had higher protein concentration than had glutelin-G_2. Water-insoluble ASG (zein-like glutelin), total glutelin-G_1 and zein isolates were highly pure. Their purities were not affected by the drying temperatures.

Precipitation of the protein content in the albumin and globulin extracts with a salt, such as ammonium sulphate, before dialysis, could improve the protein concentration of these isolates.

Table 4
Correlation coefficients (r) between the percentages of total protein sequentially extracted from whole corn kernels dried at different temperatures

	Drying temperature	Total salt-soluble proteins	Albumin	Globulin	Zein	Glutelin-G_1	Glutelin-G_2	Glutelin-G_3	Non-protein nitrogen equivalent
Total salt-soluble proteins	−0.986**								
Albumin	−0.928*	0.961**							
Globulin	−0.998***	0.981**	0.905*						
Zein	−0.997***	0.980**	0.940*	0.990***					
Glutelin-G_1	−0.825 ns	0.820 ns	−0.730 ns	0.846 ns	0.818 ns				
Glutelin-G_2	0.986**	−0.979**	−0.886*	−0.994***	−0.970**	−0.853 ns			
Glutelin-G_3	0.996***	−0.995***	−0.940*	−0.995***	−0.990***	−0.845 ns	0.990***		
Non protein nitrogen equivalent	−0.974**	0.947*	0.915*	0.961**	0.978**	0.685 ns	0.934*	-0.956*	
Unfractionated protein	0.928*	−0.869 ns	−0.740ns	−0.946*	0.919*	0.884*	0.937*	0.913*	−0.869 ns

ns, not significant; *, **, ***=$P < 0.05$, 0.01, 0.001, respectively, $n = 15$.

Table 5
Percentage of proteins contained in each isolate on dry basis

	T0	54	80	110	130
Albumin	48.7 ± 2.3	35.3 ± 0.3	30.8 ± 1.9	20.5 ± 2.9	14.8 ± 0.1
Globulins	66.7 ± 2.6	57.2 ± 3.4	42.5 ± 1.2	32.7 ± 5.9	24.2 ± 1.0
Total salt-soluble proteins	76.3 ± 1.6	51.1 ± 1.8	37.9 ± 2.6	26.1 ± 0.6	18.6 ± 5.3
Zein	99.2 ± 1.6	98.9 ± 1.0	98.7 ± 3.5	99.9 ± 2.4	98.2 ± 3.9
Total glutelin-G_1	99.3 ± 3.8	102.3 ± 1.2	102.3 ± 2.7	103.4 ± 1.0	97.0 ± 4.4
Water-insoluble ASG	100.8 ± 5.2	98.3 ± 7.1	99.9 ± 5.5	99.3 ± 4.7	102.6 ± 3.4
Glutelin-G_2	18.8 ± 2.1	24.3 ± 1.0	32.2 ± 3.4	34.7 ± 2.5	48.5 ± 3.2
Glutelin-G_3	68.6 ± 2.7	68.8 ± 1.8	73.3 ± 3.4	78.2 ± 4.7	74.1 ± 2.0

Table 6 shows that the concentrations of albumin, globulin, and glutelin-G2 isolated were significantly influenced by the drying temperature. Except for zein, glutelin-G_1 and glutelin-G_2, the purities of isolates depended on the relative amount of proteins extracted from the whole corn kernel before dialysis.

The functional properties of corn protein preparations reflect the composition of the sample, the nature and the reactivity of proteins, their native structure and interactions with the non-protein components (Lin & Zayas, 1987). According to previous results, that drying temperatures may affect the functional properties of corn protein preparations.

Table 6
Correlation coefficients between the isolate purity, the drying temperature and the percentage of total protein content in the extract before dialysis

Isolate	Drying temperature	% of total protein in the extract before dialysis
Albumin	−0.946*	0.929*
Globulin	−0.977**	0.972**
Total Salt-soluble protein	0.917*	0.961**
Zein	0.169 ns	0.235 ns
Glutelin-G_1	0.459 ns	0.592 ns
Glutelin-G_2	−0.952*	0.779 ns
Glutelin-G_3	−0.814 ns	0.950*

ns, not significant; *, **=$P < 0.05$, 0.01, respectively, $n = 15$.

3.4. Electrophoretic patterns of proteins isolated from dried corn kernels

SDS-PAGE showed the disappearance of some water and salt-soluble polypeptides at high drying temperatures (Figs. 2 and 3). Except for the decrease in intensity of some high molecular mass (Mw) bands of total salt-soluble proteins, the use of 2-ME did not enhance differences between the patterns obtained under reduced and non-reduced conditions.

The loss of salt-soluble proteins increased above 80 °C. At 80 °C, a band localized approximately at 26,500 Mw was present in the electrophoretic patterns, while most of the high molecular mass bands disappeared. This band was attributed to extracted globulin.

The comparison of electrophoretic patterns of albumin and globulin (Fig. 3) showed the disappearance of high molecular mass bands of albumin, while globulin patterns showed no significant decrease of band intensity up to 80 °C. The total disappearance of high molecular mass bands of globulin from corn dried above 80 °C corroborates the abrupt decrease of nitrogen extracted as salt-soluble material after the removal of albumin.

The electrophoresis patterns of zein (Fig. 4) and glutelin-G_1 (Fig. 5) were not affected by the drying temperature, whereas the solubility of zein appeared to be affected by it. Probably, the denaturation mechanism of zein-like proteins is not significantly selective for the particular polypeptides present in these corn proteins fractions.

Reduced zein exhibited four major bands on SDS-PAGE, while the non-reduced zein patterns presented several bands with incremental subunits of approximately 22,000 and 24,000 Da (Fig. 4). This is consistent with the disulfide structure of zein previously observed by Turner, Boundy, and Dimler (1965) in starch gel electrophoresis and by Paulis (1981) and Landry (1979) in SDS-PAGE.

The SDS-PAGE pattern of glutelin-G_1 (Fig. 5) showed the two major bands initially observed in the zein pattern at 22,000 and 24,000 Da and two low molecular mass bands of approximately 17,000 and 13,000 Da. A similar major band of approximately 13,000 Da was also observed in the albumin pattern (Fig. 3).

Glutelin-G_3 gave a diffuse electrophoretic pattern and did not present a discernable electrophoretic band, for such analysis conditions.

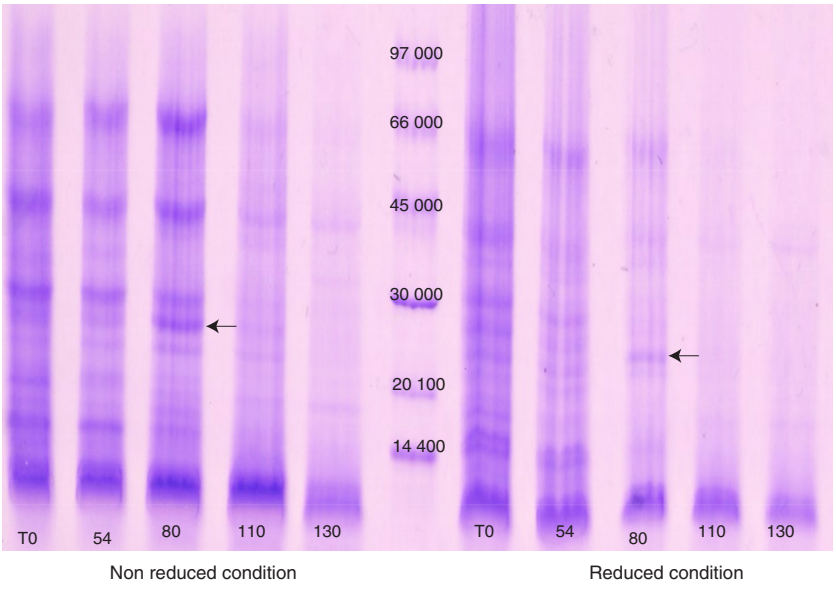

Fig. 2. SDS-PAGE of total salt-soluble proteins extracted from corn dried at different temperatures, under non-reduced (left) and reduced conditions (right); *y* axis measurement = Da.

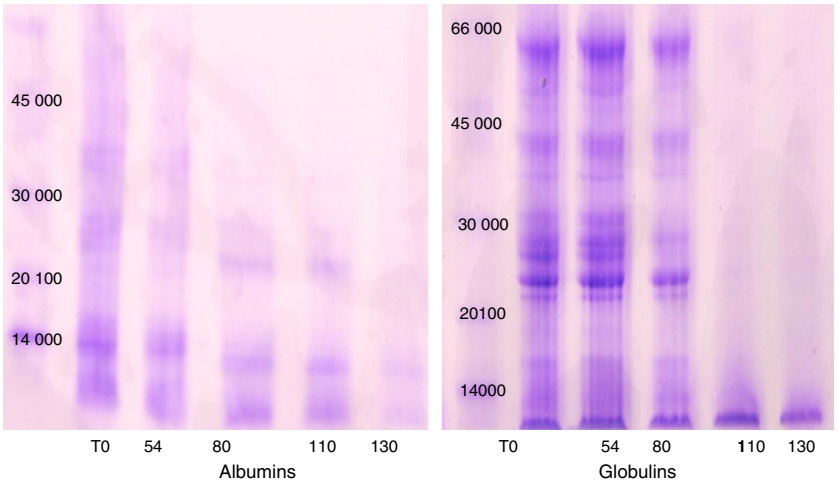

Fig. 3. SDS-PAGE of albumin (left) and globulin (right) isolates under the reduced condition; *y* axis measurement = Da.

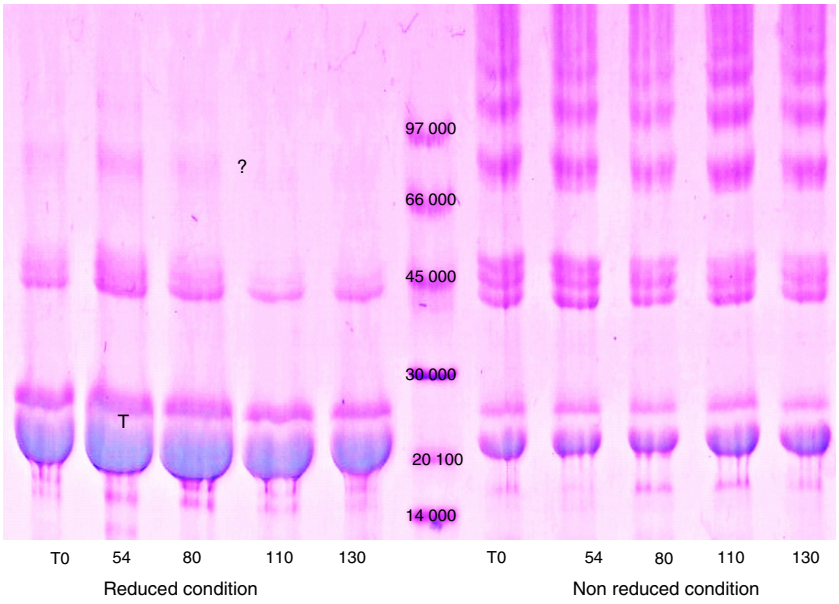

Fig. 4. SDS-PAGE of total zein extracted from whole corn dried at different temperatures, under reduced (left) and in non-reduced (right) conditions.

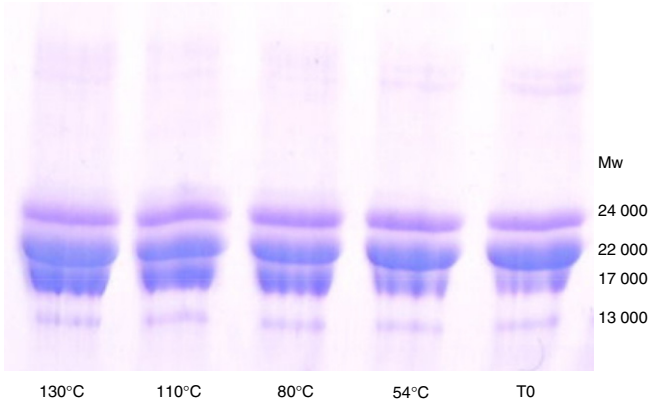

Fig. 5. SDS-PAGE of total reduced alcohol-soluble glutelin (glutelin-G_1) extracted from corn dried at different temperatures, under reduced condition.

4. Conclusion

An Osborn and Mendel type of sequential extraction of corn proteins, as modified by Landry and Moureaux (1970) and Paulis (1982) was performed in order to elucidate the influence of the drying temperature on the extraction of different corn protein groups, on the purity of dialyzed protein isolates and on the electrophoretic patterns of corn protein isolates. The globulin and the zein extractability dropped significantly when the drying temperature increased, while fractions extracted as glutelin-G_2 and glutelin-G_3 increased up to 110 °C before dropping slightly at 130 °C. The amounts of insoluble proteins rose significantly at 130 °C. The analysis of the different protein group solubilities suggested that denaturation mechanisms other than the creation of new disulfide bonds occurred during the drying of corn at high temperature. The purities of recovered albumin and globulin isolates decreased continuously while the purity of

glutelin-G_2 and G_3 isolates increased with increase of corn drying temperature. Water-insoluble glutelin-G_1 and zein isolates were highly pure, and their purities were not affected by drying temperatures. SDS-PAGE showed the disappearance of some water and salt-soluble polypeptides at high drying temperatures. The electrophoretic patterns of zein and glutelin-G_1 were not affected by the drying temperature, although the solubility of zein was changed. The denaturation mechanism of zein-like proteins is probably not selective for the particular polypeptides present in these corn protein fractions. Results reported in the present study show that changes occurred in the corn protein structure, which may influence functional properties of cornprotein-based products.

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References

Bewley, J. D., & Black, M. (1978). *Physiology and biochemistry of seeds in relation to germination*. New York: Springer-Verlag.

Eckhoff, S. R. (2004). Wet Milling. In C. Wrigley & C. E. Walker (Eds.). *Encyclopedia of grain science* (Vol. 2, pp. 225–241). Amsterdam: Elsevier Ltd.

Hall, C. W. (1980). *Drying and storage of agricultural crops*. West port, CT: AVI.

Hung, S. C., & Zayas, J. F. (1992). Protein solubility, water retention, and fat binding of corn germ protein flour compared with milk proteins. *Journal of Food Science, 57*(2), 372–376.

Jayas, D. S., & White, N. D. G. (2003). Storage and drying of grain in Canada: Low cost approaches. *Food Control, 14*, 255–261.

Landry, J. (1979). La zéine du grain de maïs: Préparation et caractérisation. *Biochimie, 61*, 549–558.

Landry, J., Delhaye, S., & Di Goiai, L. (1999). Protein distribution in gluten products isolated during and after wet-milling of maize grains. *Cereal Chemistry, 76*(4), 503–505.

Landry, J., & Moureaux, T. (1970). Hétérogénéité des Glutélines du grain de maïs: Extraction sélective et composition en acides aminés des trois fractions isolées. *Bulletin de la Societe de Chimie Biologique, 52*(10), 1021–1037.

Landry, J., & Moureaux, T. (1980). Distribution and amino-acid composition of protein groups located in different histological parts of maize grain. *Journal of Agricultural and Food Chemistry, 28*, 1186–1191.

Landry, J., & Moureaux, T. (1981). Physicochemical properties of maize glutelins as influenced by their isolation conditions. *Journal of Agricultural and Food Chemistry, 29*, 1205–1212.

Lasseran, J. C. (1973). Incidence of drying and storing conditions of corn (maize) on its quality for starch industry. *Starch, 25*(8), 257–262.

Li, J. S., & Vasal, S. K. (2004). Quality protein maize. In C. Wrigley & C. E. Walker (Eds.). *Encyclopedia of grain science* (Vol. 2, pp. 212–216). Amsterdam: Elsevier Ltd.

Lin, C. S., & Zayas, J. F. (1987). Functionality of defated corn germ proteins in a model system: Fat binding capacity and water retention. *Journal of Food Science, 52*(5), 1308–1311.

Lupano, C. E., & Añon, M. C. (1986). Denaturation of wheat proteins during drying. *Cereal Chemistry, 63*(3), 259–262.

McGuire, T. A., & Earle, F. R. (1958). Changes in solubility of corn protein resulting from three artificial drying of high-moisture corn. *Cereal Chemistry, 35*, 179–188.

Moueium, Z. A., El Tinay, A. H., & Abdalla, A. W. (1996). Effect of germination on protein fraction of corn cultivars. *Food Chemistry, 57*(3), 381–384.

Neumann, P. E., Wall, J. S., & Walker, C. E. (1984). Chemical and physical properties of proteins in wet-milled corn gluten. *Cereal Chemistry, 61*(4), 353–356.

Osborne, T. B., & Mendel, L. B. (1914). Nutritive properties of proteins of the maize kernel. *The Journal of Biological Chemistry, 18*(1), 1–16.

Pallai, E., Németh, J., & Mujumdar, A. S. (1987). Spouted bed drying. In A. S. Mujumdar (Ed.), *Handbook of industrial drying* (pp. 419–460). New York: Marcel Dekker, Inc.

Paulis, J. W. (1981). Disulfide structures of zein proteins from corn endosperm. *Cereal Chemistry, 58*(6), 542–546.

Paulis, J. W. (1982). Recent developments in corn protein research. *Journal of Agricultural and Food Chemistry, 30*, 14–20.

Paulis, J. W., James, C., & Wall, J. S. (1969). Comparison of glutelin proteins in normal and high-lysine corn endosperms. *Journal of Agricultural and Food Chemistry, 17*(6), 1301–1305.

Paulis, J. W., & Wall, J. S. (1969). Albumins and globulins in extracts of corn grain parts. *Cereal Chemistry, 46*, 263–273.

Paulis, J. W., & Wall, J. (1977). Comparison of the protein composition of selected corns and their wild relatives, teosinte and tripsacum. *Journal of Agricultural and Food Chemistry, 25*(2), 265–270.

Sodek, L., & Wilson, C. M. (1971). Amino acid composition of proteins isolated from normal, opaque-2, and floury-2 corn endosperms by a modified Osborne procedure. *Journal of Agricultural and Food Chemistry, 19*(6), 1144–1150.

Townsend, A.-A., & Nakai, S. (1983). Relationships between hydrophobicity and foaming characteristics of proteins. *Journal of Food Science, 48*, 588–594.

Turner, J. E., Boundy, J. A., & Dimler, R. (1965). Zein: A heterogeneous protein containing disulfide-linked aggregates. *Cereal Chemistry, 42*, 452–461.

Wall, J. S., James, C., & Donaldson, G. L. (1975). Corn proteins: Chemical and physical changes during drying of grain. *Cereal Chemistry, 52*, 779–790.

Weller, C., Paulsen, M. R., & Steinberg, M. P. (1988). Correlation of starch recovery with assorted quality factors of four corn hybrids. *Cereal Chemistry, 65*(5), 392–397.

Wight, A. W. (1981). Changes in properties of some maize cultivars associated with artificial drying at elevated temperatures. Part II. Protein solubility and other properties in relation to milling quality. *Starch, 33*(5), 165–168.