Isolation and Quantitation of Zeins in *Waxy* and *Amylose-Extender* and Wild Flint and Dent Maize Endosperm Using a New Solvent Sequence for Protein Extraction

Jacques Landry,*,† Sonia Delhaye,† Christelle Philippeau,‡ and Brigitte Michalet-Doreau‡

Laboratoire de Chimie Biologique, CBAI, INRA, 78850 Thiverval Grignon, France, and Unité Digestion Microbienne, SRNH, INRA Theix, 63122 St. Genès Champanelle, France

Protein distribution in endosperm of maize grains differing by their texture, flint or dent, and by their genotype, wild or *waxy* or *amylose-extender*, was examined by the successive use of 0.5 M NaCl, 0.5 M NaCl plus 0.6% 2-mercaptoethanol (2ME) at neutral and then alkaline pH, and 55% 2-propanol plus 0.6% 2ME as extractants. Proteins extracted in the presence of 2ME were characterized by their size polymorphism and amino acid composition. Proteins isolated with NaCl plus 2ME at neutral pH corresponded with a mixture of γ-zein (27 kDa) and glutelin-like proteins. Proteins isolated with NaCl plus 2ME at pH 10 were a mixture of γ-zeins (27 and 16 kDa) and β-zeins (14 kDa). Alcohol-soluble proteins consisted of α-, β-, and δ-zeins, α subunits being predominant. Zein quantitation was improved by weighing the nitrogen percentage of extracts by their zein content, as estimated from the data on amino acid composition. The data reported by Wolf et al. (*Cereal Chem.* **1975**, *52*, 765) were integrated to the results of this work to suggest the occurrence of an inverse correlation between amylose in starch and zeins in proteins.

Keywords: *Maize; endosperm; proteins; zeins; amylose level*

INTRODUCTION

Zeins form the storage proteins located in the protein bodies of endosperm of maize grains. According to Wilson (1991) they can be classified as (I) α-zeins, made up of major polypeptides with relative molecular masses (M_r) of 19 and 22 kDa, soluble in aqueous alcohols; (2) β- and δ-zeins, consisting of polypeptides with respective M_r values of 14 and 10 kDa, soluble in aqueous alcohols in the presence of reducing agent; and (3) γ-zeins, composed of polypeptides with M_r values of 16 and 27 kDa, soluble in both aqueous and alcoholic solvents in the presence of salt and reducting agent.

The classical procedures used for fractionating protein endosperm are based on sequential solvent extraction. Using such procedures, zeins have been categorized into two subsets as unreduced α-zeins and a mixture of reduced α-, β-, γ-, δ-zeins, respectively termed Z_1 and Z_2 by Sodek and Wilson (1971) or zein and alcohol-soluble reduced glutelin by Paulis and Wall (1977). Landry and Moureaux (1970) have classified them into three subsets as unreduced α-zeins, a mixture of reduced α-, β-, δ-zeins, and reduced γ-zeins, respectively referred to as zein and G_1- and G_2-glutelins. The relative importance of each zein fraction was evaluated using a Kjeldahl procedure.

The G_2-glutelins (or fraction FIV), isolated according to the Landry−Moureaux procedure, were found to be a mixture of polypeptides corresponding to γ-zeins (27 kDa) and to glutelin-like proteins that were extracted at pH 3 and 10, respectively (Landry and Moureaux, 1981).

Furthermore, G_2-glutelins were partially coextracted with G_1-glutelins if salt was not removed from meal exhaustively (Landry et al., 1983) (this has been rediscovered in 1990 by Wallace et al.) and could be isolated from the latter as a water-soluble fraction. G_2-glutelins can also be solubilized after salt extraction and before alcohol extraction with 0.5 M NaCl plus 0.6% (v/v) 2-mercaptoethanol (2ME) using solvent sequence B (Landry and Moureaux, 1970) and were used by Wilson et al. (1981) to isolate reduced soluble proteins. Those proteins were associated with protein bodies (Vitale, 1982) and located at their periphery (Ludevid et al., 1984).

These observations prompted us to use successively aqueous solutions of NaCl, NaCl plus 2ME (nonbuffered and then buffered at pH 10), and 2-propanol plus 2ME with the aim of determining the protein distribution and of better isolating zein subsets in endosperm of six maize samples differing by their genotype and by their texture. The selectivity of such a sequence was assessed by determining the size polymorphism and the composition of extracted proteins using sodium dodecyl sulfate−polyacrylamide gel electrophoresis (SDS−PAGE) and amino acid analysis, respectively.

MATERIALS AND METHODS

The majority of experimental conditions are described by Philippeau et al. (1998).

Grains. The six samples of maize (*Zea mays* L.) originated from two cultivars differing in the texture of the endosperm, flint or dent. Morphological characteristics and biochemical composition of grains were reported by Phillippeau et al. (1998). Grains were hand dissected for endosperm isolation. Endosperms were ground through a 2 mm sieve in a hammer mill, and meal was defatted with hexane.

Extraction of Proteins. The selective extraction of endosperm proteins was conducted on a 500 mg sample according

* Author to whom correspondence should be addressed (fax 033-1-30-81-53-73; e-mail landry@grignon.inra.fr).
† Laboratoire de Chimie Biologique.
‡ Unité Digestion Microbienne.

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Table 1. Protein Distribution in Six Endosperm Samples Differing by Their Texture and Genotype[a]

		flint			dent		
step	extractant[b]	+	*wx*	*ae*	+	*wx*	*ae*
1	0.5 M NaCl	3.4 (0.17)	6.7 (0.11)	11.1 (0.07)	10.2 (0.19)	8.0 (0.09)	I3.0 (0.11)
2	0.5 M NaCl + 2ME	9.1 (0.28)	8.1 (0.74)	9.9 (0.45)	9.3 (0.46)	10.8 (0.55)	9.3 (0.98)
3	0.5 M NaCl + 2ME (pH 10)	8.2 (0.58)	7.7 (0.76)	10.9 (0.80)	4.9 (0.54)	6.5 (0.27)	7.6 (0.25)
4	55% 2PrOH + 2ME	67.4 (1.00)	66.1 (1.21)	48.4 (1.86)	62.0 (1.20)	62.1 (0.06)	52.5 (4.50)
5	(insoluble)	11.9 (0.20)	11.4 (0.10)	19.7 (0.40)	13.7 (0.42)	12.6 (0.66)	17.6 (2.70)
	total protein % db[c]	13.8	11.7	11.1	14.8	13.2	13.9

[a] Percentage of recovered endosperm proteins from duplicate extractions (standard deviation). [b] Extractants: 2ME, 0.6% (v/v) 2-mercaptoethanol; 2PrOH, 2-propanol. [c] N × 6.25, dry basis.

to sequence B of the Landry and Moureaux (1970) method, slightly modified. It involved the successive use of 0.5 M NaCl at 4 °C (two times), 0.5 M NaCl plus 0.6% (v/v) 2ME at 20 °C (two times), 0.5 M NaCl plus 0.5 M sodium acetate plus 0.6% 2ME buffered at pH 10 at 20 °C (one time), and 55% (w/w) 2-propanol plus 0.6% 2ME at 20 °C (three times). The time of contact of solvent with meal was 30 min. Extractants were isolated from solid particles by centrifugation at 30000*g* for 15 min. For each solvent, supernatants were combined, and the nitrogen content was determined using a Kjeldhal method (AOAC, 1990).

Protein Characterization. Sodium dodecyl sulfate−polyacrylamide gel electrophoresis (SDS−PAGE) was performed according to the method of Laemmli (1970), using precast 4−20% polyacrylamide gels.

Amino acid compositions were determined by hydrolyzing extracts directly or after they have been subjected to performic oxidation with constant-boiling HCl under vacuum for 24 h (18 h for oxidized samples). Phenylthiocarbamyl (PTC) derivatives of released amino acids were separated using reverse-phase high-performance liquid chromatography and quantitated from their absorbance at 254 nm (Bidlingmeyer, 1984). Tryptophan was determined according to the method of Landry and Delhaye (1992) as refined by Fontaine et al. (1998).

RESULTS AND DISCUSSION

Protein Distribution in Endosperm. Table 1 gives the proportions of protein isolated at each step, expressed as a percentage of recovered total proteins. Protein recovery varied from 95.0 to 103.0%. Salt-soluble proteins constituted from 3.4 to 13.0% and averaged 8.7%. Percentages of proteins extracted in the presence of salt and reducing agent were relatively independent of genotype, ranging from 8.1 to 9.9% and averaging 9.4%. Percentages of proteins solubilized at pH 10 with the reducing agent were surprisingly large considering that only one extraction was performed at this step. They ranged from 4.9 to 10.9% and averaged 7.6%. Alcohol-soluble proteins were predominant, averaging 64.4% for wild and *waxy* maizes and 50.5% for the *amylose-extender* genotype. Residual proteins, made up of true glutelins, were the second major fraction, averaging 12.4% (+ and *wx*) and 18.7% (*ae*).

Electrophoretic Analysis. The distribution of relative molecular masses of proteins extracted in the presence of reducing agent with salt, at neutral and then alkaline pH, and with alcohol, is shown in Figure 1, which depicts the patterns of the extracts isolated from wild and *wx* flint maizes.

The proteins isolated using 2ME and not buffered salt ("neutral salt" extract) (Figure 1, lanes 1 and 5) were γ-zein (27 kDa) as main polypeptide, followed by two polypeptides with M_r values of 50 and 12 kDa, the latter value being estimated from that of 10 kDa attributed to δ-zein present in alcoholic extract. Some minor bands are also seen. These indicate the probable occurrence

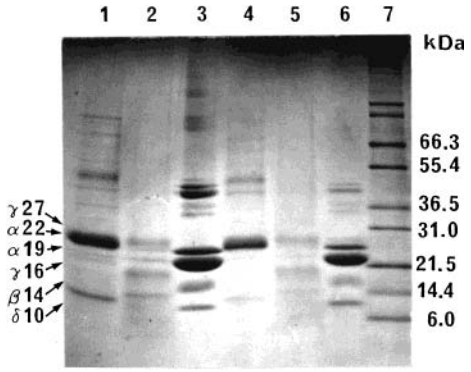

Figure 1. SDS−PAGE of proteins extracted in the presence of 2ME and 0.5 M NaCl not buffered (lanes 1 and 4), 0.5 M NaCl buffered at pH 10 (lanes 2 and 5) and 55% 2-propanol (lanes 3 and 6) from wild (lanes 1−3) and *waxy* (lanes 4−6) maizes. The positions and molecular masses of protein standards are indicated on the right. The positions of α-, β-, γ-, and δ-zeins are shown on the left.

of albumins and gobulins incompletely extracted in the absence of reducing agent.

The pattern of extracts isolated in the presence of salt and the reductant at pH 10 ("alkaline salt" extract) (Figure 1, lanes 2 and 4) consisted of (1) a doublet, the slowest and main band of which would be γ-zein (27kDa) and having a minor band displaying the same mobility as α-zein (22kDa); (2) a minor band migrating at the level of α-zein (19kDa); (3) a band that was considered as γ-zein (16kDa) because it migrated slightly more slowly than that of β-zein (14kDa) found in alcoholic extract; and (4) a 12 kDa band.

Alcoholic extracts contained α- (22 and 19kDa), β-(14kDa), and δ-zeins (10kDa) with some oligomeric forms, the occurrence of which was probably related to the interval of time elapsed between extraction and electrophoresis (Figure 1, lanes 3 and 6).

It is interesting to compare the electrophoretic data of Figure 1 with those reported by Landry et al. (1983), who isolated an extract using 55% 2-propanol plus 1.2% 2ME from endosperm meal previously treated by saline and then alcoholic solutions, in the absence of a reducing agent (Figure 2). This extract, referred to as G_1-glutelins by Landry and Moureaux (1970), contained all of the bands evident in the patterns of the three extracts depicted in Figure 1, excluding the 12 kDa band. Further dialysis of the G_1 extract against dilute acetic acid led to the isolation of two fractions. The first one, termed G_1p-insoluble and havinge a SDS−PAGE pattern that resembled that of the alcohol extract isolated in the present study (Figure 1, lanes 3 and 6) with additional minor bands corresponding to γ-zeins (27 and 16kDa), coprecipitated with alcohol-soluble proteins. The pattern of the other fraction, termed G_1s soluble,

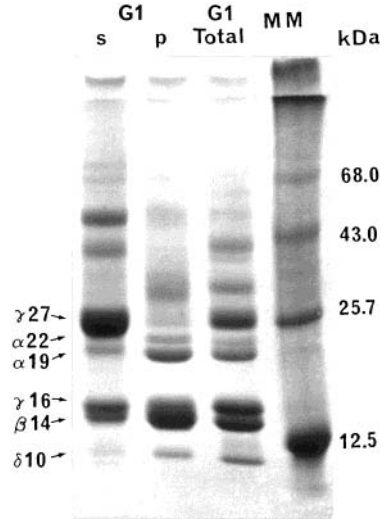

Figure 2. SDS−PAGE of proteins isolated with 55% 2-propanol + 1.2% 2ME (G_1-glutelins) from endosperm meal (W64A maize) previously treated with 0.5 M NaCl, H_2O, and 55% 2-propanol and then fractionated by dialysis of extract into soluble G_1s and insoluble G_1p. The positions and molecular masses of protein standards are indicated on the right. The positions of α-, β-, γ-, and δ-zeins are shown on the left. Data are from Landry et al. (1983).

corresponded to the superimposition of those obtained for the two salt extracts of the present study (Figure 1, lanes 1 and 2 and lanes 4 and 5), excluding the 12 kDa polypeptide. This polypeptide was found in the alcoholic extracts when they were isolated from an SDS extract of total proteins according to the procedure of Wallace et al. (1990).

The present data together with those reported by Landry et al. (1983) elicited the amphiphilic character of γ-zeins (27 and 16kDa) and were consistent with previous observations. An alcohol-soluble polypeptide of M_r 16 kDa was first considered as β-zein by Esen (1987) and then later as γ-zein.

Amino Acid Analysis. The amino acid compositions reported in the tables for a given extract correspond to the mean composition of extracts obtained from the six endosperm samples.

The amino acid composition of "neutral salt" extract is given in Table 2 together with data reported in the literature for the sake of comparison. First, the composition appeared to be identical to that given by Landry and Moureaux (1970) for an extract obtained under the same conditions but from whole grain, and its amino acid contents have been recalculated to take into account more accurate contents for cysteine and methionine. This identity, despite the use of two different procedures for amino acid analysis, was in agreement with the previous claim of Landry and Moureaux (1980) that this fraction was located in endosperm.

From electrophoretic data the "neutral salt" extract appeared as a mixture of γ-zein (27kDa) with polypeptides of higher or lower molecular masses. For comparison, Table 2 lists the amino acid composition of two polypeptides of 28 kDa (fractions I and II) isolated by Vitale et al.(1982) and another of 58 kDa isolated by Ludevid et al. (1984). The "neutral salt" extract displayed an amino acid composition similar to that of a mixture made up of 28 and 58 kDa polypeptides in equal proportions, which fitted with the electrophoretic data.

The mean amino acid composition of the "alkaline salt" extract (Table 3) was different from that of G_1p proteins isolated by Landry et al. (1983), contrary to what the electrophoretic data suggested. The high content of glycine, histidine, tyrosine, and tryptophan would be in line with the presence of a polypeptide similar in composition to γ-zein (16kDa). However, the high content of aspartate, methionine, and tyrosine and the low level of cysteine indicated the occurrence of other polypeptides. Actually, there was a good similarity

Table 2. Amino Acid Composition of Proteins Extracted with 0.5 M NaCl plus 0.6% 2ME ("Neutral Salt" Extract)[a]

	sample[b]					
	S1	S2	S3	S4	S5	S6
aspartate	3.1 (8.2)	2.2	0	0.6	6.7	3.5
glutamate	19.8 (5.7)	19.8	16.2	16.4	21.8	19.1
serine	5.6 (5.0)	5.3	3.9	4.5	7.5	5.8
glycine	7.0 (4.6)	7.7	6.8	6.8	9.5	8.0
histidine	5.7 (7.4)	5.3	7.5	8.9	5.6	6.9
arginine	3.5 (4.4)	3.4	2.6	2.4	2.2	2.4
threonine	3.9 (3.0)	4.9	4.4	4.6	4.0	4.2
alanine	6.3 (5.1)	6.6	5.1	5.2	6.3	5.7
proline	15.9 (14.7)	17.4	23.3	19.2	12.7	17.0
tyrosine	2.6 (7.8)	2.4	1.8	3.3	0.6	1.6
valine	6.4 (2.1)	6.3	7.2	9.5	5.0	6.7
methionine	1.3 (31.7)	1.2	0.4	0.4	0.6	0.5
cystine	4.9 (8.7)	5.0	7.9	6.7	9.0	8.1
isoleucine	2.7 (7.5)	2.4	1.7	1.5	3.0	2.3
leucine	8.7 (5.1)	8.4	9.3	8.8	5.4	7.2
phenylalanine	1.7 (9.4)	1.7	1.0	1.3	2.0	1.6
lysine	1.6 (12.0)	1.8	0	0	3.0	1.5
tryptophan	0.3 (26.7)	nd[c]	nd	nd	nd	nd

[a] Mole percent. [b] Samples: S1, mean composition and coefficient of variation in percent of six extracts isolated from the six samples examined in this study; S2, the same extract isolated from grain by Landry and Moureaux (1970) for which the amino acid composition has been recalculated by taking the content of sulfur amino acids into account; S3 and S4, fractions I and II of 28 kDa isolated from protein bodies by Vitale et al. (1982); S5, fraction of 58 kDa isolated by Ludevid et al. (1985); S6, mixture of S3, S4, and S5 accounting for 25, 25, and 50%, respectively. [c] Not determined.

Table 3. Amino Acid Composition of Extracts Isolated with 0.5 M NaCl plus 0.6% 2ME Buffered at pH 10 ("Alkaline Salt" Extract)[a]

	sample[b]				
	S1	S2	S3	S4	S5
aspartate	2.8 (10.1)	2.4	2.5	2.7	2.9
glutamate	18.9 (2.5)	20.1	17.9	20.1	19.1
serine	58 (4.2)	5.0	6.0	5.9	6.0
glycine	8.5 (5.6)	8.1	9.0	9.6	5.7
histidine	3.0 (4.7)	1.3	1.2	1.1	3.3
arginine	3.3 (7.9)	2.8	2.4	3.1	1.9
threonine	3.3 (4.9)	3.0	3.9	6.1	5.5
alanine	10.2 (6.4)	12.1	10.4	13.9	10.5
proline	10.6 (3.8)	14.1	11.9	5.4[c]	9.0[c]
tyrosine	5.0 (7.3)	6.7	4.7	6.5	3.4
valine	5.6 (4.1)	3.4	3.4	2.8	5.3
methionine	5.0 (10.8)	7.1	5.3	5.9	2.7
cystine	1.2 (30.0)	nd[d]	tr[e]	4.2	2.7
isoleucine	2.0 (14.1)	1.1	1.3	1.0	2.7
leucine	10.1 (2.7)	11.1	11.6	9.9	13.6
phenylalanine	2.9 (8.3)	1.6	2.8	1.3	3.8
lysine	1.3 (16.2)	0.1	0.3	0.5	2.0
tryptophan	0.4 (15.2)	nd	nd	nd	nd

[a] Mole percent. [b] Samples: S1, mean composition and coefficients of variation in percent of six extracts isolated from the six endosperm samples examined in this study; S2, cryoprecipited zein II isolated by Melcher and Fraij (1980); S3, zein fraction of 14 kDa isolated by electrophoresis by Gianazza (1978); S4 and S5, chromatographic fractions 1 and 3A, respectively, isolated from water-insoluble alcohol-soluble glutelin by Paulis and Bietz (1986). [c] Doubtful values. [d] Not determined. [e] Traces.

Table 4. Amino Acid Composition of Alcohol-Soluble Proteins[a]

	sample[b]	
	S1	S2
aspartate	5.0 (18.0)	5.4
glutamate	21.0 (3.0)	22.0
serine	6.8 (7.6)	6.9
glycine	2.3 (11.0)	2.1
histidine	1.8 (9.3)	0.9
arginine	1.6 (48.1)	1.0
threonine	2.8 (9.6)	3.0
alanine	14.3 (4.3)	13.8
proline	9.7 (14.0)	9.7
tyrosine	3.5 (9.3)	3.5
valine	3.2 (9.3)	3.6
methionine	2.4 (17.6)	1.0[c]
cystine	0.9 (11.8)	0.3[c]
isoleucine	2.8 (7.1)	2.9
leucine	17.5 (1.8)	18.7
phenylalanine	4.3 (5.3)	5.1
lysine	0	0.1
tryptophan	0.06 (33.0)	nd[d]

[a] Mole percent. [b] Samples: S1, mean composition and coefficient of variation of six extracts isolated from the six endosperm samples examined in this study; S2, extract isolated with 55% 2-propanol (without 2ME) from endosperm of commercial hybrid by Landry and Moureaux (1980). [c] Underestimated values due to the absence of performic oxidation. [d] Not determined.

of composition between the "akaline salt" extract and the cryoprecipitated fraction of proteins extracted with aqueous ethanol plus 2ME and which contained 12, 13, 15, and 16kDa bands (Melcher and Fraj, 1980) or the zein fraction of 14 kDa isolated by electrophoresis (Gianazza, 1977) or fraction 1 isolated from water-insoluble alcohol-soluble glutelins (wi-ASG) subjected to reverse-phase high-performance liquid chromatography (Paulis and Bietz, 1986). Another fraction (3A) isolated by the same authors was rich in lysine (2%). Its presence in "alkaline salt" extract would allow one to explain the content of lysine.

From the above background the "alkaline salt" extracts would consist of polypeptides belonging to β-zein mainly, but not detected by electrophoresis. These, although hydrophobic by nature, were extracted in aqueous medium at pH 10 due to their high level of tyrosyl groups [8.8 mol % according to amino acid sequence deduced from cDNA (Pedersen et al., 1986)] ionized at this pH. The addition of SDS buffered at pH 6.8 (sample buffer) to extract would result in precipitation of proteins not complexed with SDS due to electrostatic repulsion of anionic detergent by negatively ionized tyrosyl groups, which would explain the absence of these proteins in electrophoretic patterns. Furthermore, the presence of tryptophan in significant proportion was in agreement with the occurrence of γ-zein (16kDa), detected in the electrophoretic pattern and which contains one tryptophanyl residue according to the sequence deduced from cDNA (Prat et al., 1987).

The average amino acid composition of alcoholic extracts (Table 4) was consistent with that of a mixture of α-, β-, and δ-zeins. It displayed a great similarity, if not identity, with the amino acid composition of the alcoholic extract isolated from the endosperm in the absence of a reducing agent, which suggested the predominance of α subunits.

Quantitation of Zeins. From the data shown in Table 1 and the electrophoretic patterns of Figure 1, the percentage of total zeins can be approximated by summing the percentages of nitrogen extracted by salt plus 2ME at neutral and then at alkaline pH and by alcohol plus 2ME, that is, at steps 2, 3, and 4. Zeins ranged from 69.4 to 84.7% of total recovered N. However, amino acid analyses emphasized that the "neutral salt" extract was a mixture of zeins and glutelin-like proteins in equal proportions. Furthermore, according to its lysine content, the "alkaline salt" extract would be a mixture of zeins and proteins that were assumed to be true glutelins, whose lysine content averaged 5.4 mol % (Wilson, 1983), and which account for 25% of the total. With these estimations taken into consideration, total zeins would amount to 61.5−78.1% (Table 5), pointing out a lower proportion of zeins with the presence of the *amylose-extender* gene.

Table 5. Quantitative Estimation of Zeins in the Six Endosperm Samples Examined in This Study[a]

	sample[b]					
	flint			dent		
	+	*wx*	*ae*	+	*wx*	*ae*
E2+ E3 + E4[c]	84.7	81.9	69.4	76.2	79.4	69.4
0.5 E2 + 0.75 E3 + E4[d]	78.1	75.9	61.5	70.3	72.4	62.9

[a] Percent of total nitrogen recovered. [b] Samples: +, *wx*, and *ae*, wild, *waxy*, and *amylose-extender* genotypes. [c] Sum of nitrogen percntages pertaining to the extracts isolated at steps 2−4. [d] Sum of nitrogen percentages pertaining to the same extracts but weighted by their relative content of zeins, as estimated from data about amino acid compositions (see the text).

Table 6. Quantitative Estimation of Zeins in the Six Endosperm Samples Examined by Wolf et al. (1975)[a]

	sample[b]					
	W64A	W64A *ae*	dent +	amylo-maize 5	amylo-maize 7	amylo-maize 8
zeins est 1[c]	80.7	74.4	77.2	64.4	63.4	57.5
zeins est 2[d]	77.1	74.3	77.1	67.1	65.7	58.6
est 1/est 2	1.05	1.00	1.00	0.96	0.96	0.98

[a] Percent of total nitrogen. [b] Samples: amylosemaizes 5, 7, and 8, high-amylose hybrids, each with starch of a different amylose level, designated classes 5, 7, and 8. [c] Zeins (Z) were estimated from the percentages of proteins alcohol-soluble (Na) and residual (Nr) according to the equation $Z = Na + Nr(1 - [Kr/7])$, where Kr and 7 were the respective contents of lysine in the residue and in true glutelins, expressed as grams per 100 g of protein. [d] Estimation according to the equation $Z = 100(1 - [Ke/7])$, where Ke was the lysine content of endosperm (grams per 100 g of protein).

Wolf et al. (1975) have reported the effect of the *amylose-extender* gene on the protein content of maize endosperm using sequential extraction with 0.5 M NaCl followed by 70% ethanol. Integrating the data of these workers with the above-mentionned results involves estimation of the unextracted zeins present in the residue. These can be evaluated from the lysine content of the residue and the two following assumptions: (1) residual proteins are a mixture of lysine-free zeins and lysine-rich non-zein proteins; (2) the lysine content of non-zeins corresponds to that of true glutelins (Landry−Moureaux fraction V), estimated as 7 g per 100 g of protein by Misra et al. (1975) or as 5.4 mol % by Wilson (1983). The same assumptions can be used for calculation of total zeins from the lysine content of endosperm. Both estimations (Table 6) are similar or provide similar results. Furthermore, such a proceeding revealed that endosperm from W64A maize would contain 78.9% of total nitrogen as zeins. It is noteworthy that Landry et al. (1983) found 81.6% of total N as lysine-free zeins (corresponding to zeins plus G_1-glutelins plus "G_2-acetate") from the horny part of W64A endosperm,

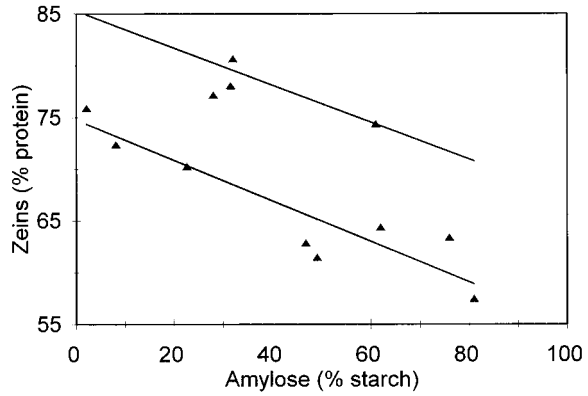

Figure 3. Percentage of zeins in proteins as a function of percentage of amylose in starch. Data relative to zeins come from Tables 5 and 6 and those relative to amylose from Wolf et al. (1975) and Philippeau et al. (1998).

which has a protein content of 13.75%. The revisited results confirmed lower proportions of zeins for amylomaizes.

Because the presence of starch-forming mutant genes in endosperm, such as *waxy* and *amylose-extender*, alters the amylose content of starch and affects the accumulation of zein, as isolated using aqueous alcohol in the absence of reducing agent (Tsai, 1984), it is interesting to seek confirmation of whether a relationship existed between the percentages of zeins and amylose. Figure 3 shows the percentage of zeins in total protein as a function of that of amylose in starch. The 12 data points fall into three groups: (i) 3 were fitted with a straight line having for an equation zeins % = 85.3 − 0.179[amylose %] with $r^2 = 0.88$ and would correspond to flint-type maizes; (ii) 8 points were fitted with another straight line having for an equation zeins % = 74.8 − 0.196[amylose %] with $r^2 = 0.87$; (iii) a unique point was located between the two lines, which could be the upper and lower borders of a linear regression, the equation of which would be zeins % = 80 − 0.2[amylose %]. A further study involving flint−dent maizes would allow one to better specify such a correlation.

CONCLUSIONS

The results obtained in this investigation provided some practical as well as basic information about endosperm proteins:

(i) The selectivity of sequence involving the use of salt plus reducing agent at neutral and alkaline pH before extraction of alcohol-soluble proteins was lower compared with the classical sequence D of the Landry−Moureaux procedure (1970). Overestimations of zeins by 9% for the former and 4% for the latter can be calculated from the data of present work (Table 5) and those of Landry and Moureaux (1981), respectively. A more extensive study on the influence of pH of extractant on the characteristics of isolated proteins could improve selectivity.

(ii) It was possible to to extract β- and γ-zeins (16 kDa) at moderately alkaline pH with respect to that of 0.2 M NaOH customarily employed to solubilize Osborne glutelins, defined as proteins not extracted by using water followed by salt then alcohol solutions (Osborne, 1897) and to precipitate β-zeins at acid pH. 2ME buffered at pH 10 was used after water, 70% ethanol, and 0.5 M NaCl were unable to isolate β-zeins, as can be seen from the amino acid composition of the extract (Robutti et al., 1974).

(iii) Data relative to the use of the Osborne method have been exploited for a more accurate quantitation of zeins by taking into account the lysine content of glutelins, regarded as a mixture of lysine-free unextracted zeins and lysine-rich true glutelins.

(iv) The existence of an inverse correlation between amylose in starch and zeins in protein and between carbohydrate and protein accumulation has been found.

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