NOTE

Comparison of Extraction Methods for Evaluating Zein Content of Maize Grain

JACQUES LANDRY[1]

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In 1970 Landry and Moureaux reported a procedure that allowed five protein fractions (F) to be isolated from maize grains by sequential extraction. Fraction I (FI), corresponding to albumins and globulins, was extracted from defatted meal with NaCl 0.5*M*. Fraction II (FII) was made up of zeins extracted with 55% (p/p) isopropanol after the removal of albumins, globulins, and salt. Fraction III (FIII), isolated in the presence of 55% isopropanol and 0.6% (p/v) 2-mercaptoethanol (2ME), consisted of cross-linked zein and proteins close to zein in amino acid composition. These were alcohol-soluble glutelins called G_1. Fraction IV (FIV), isolated in the presence of 0.5*M* NaCl and 0.6% 2ME buffered at pH 10, included proteins rich in basic amino acids, especially histidine. They were salt-soluble glutelins called G_2. Fraction V (FV), isolated in the presence of 0.5% (p/v) sodium dodecyl sulfate and 0.6% 2ME buffered at pH 10, was composed of true glutelins. At first, FIV was considered with FI and FV as salt-soluble proteins (Landry and Moureaux 1970). But it was put together with FII and FIII as endosperm specific proteins by Landry and Moureaux (1976, 1980). Such a regrouping was supported by occurrence of proteins rich in histidine, termed water-soluble, alcohol-soluble glutelins among the proteins isolated with 70% ethanol, 0.5% sodium acetate and 0.1*M* 2ME (Paulis and Wall 1977) and G_2 glutelins in protein bodies made up primarily of zein and G_1 glutelins (Vitale et al 1982, Ludevid et al 1985).

Wallace et al (1990) developed an isolation procedure for total zein involving the extraction of nearly all of proteins of maize endosperm with 1% sodium dodecyl sulfate and 2% 2ME buffered at pH 10 and the subsequent precipitation of nonzein proteins by adding ethanol to a final concentration of 70% (v/v). They claimed it had a higher yield when compared with that of the Landry Moureaux (LM) method. The same was concluded by Hamaker et al (1995).

This note is intended to show that the superiority of Wallace's procedure in the determination of total zein lies in the fact that FIV which contains γ-zein, was omitted in the comparison with LM method.

MATERIAL AND METHODS

The maize used in the present study was W64A inbred. Endosperm was isolated manually as described by Landry and Moureaux (1980). Proteins of whole grains and endosperm were sequentially extracted using the method of Landry and Moureaux (1970), involving an exhaustive removal of salt by washing meal with water before alcoholic extraction. If this step is omitted, FIV is partially coextracted with FIII, which leads one to conclude to incomplete extraction of γ-zein using The LM technique (Wallace et al 1990). Nitrogen was assayed with a semi-micro Kjeldahl method.

RESULTS AND DISCUSSION

Table I reports the contents of fractions FII+FIII and FII+FIII+FIV as evaluated in the present work and others, together with the content of alcohol-soluble nitrogen (ASN) according to Wallace's procedure. Note that ASN is made up of alcohol-soluble proteins and nonprotein nitrogen. Irrespective of the origin of works, the percentages of total nitrogen extracted in FII+FIII were similar when grains or endosperms originated from the same genotypes or from different genotypes. The ratio of nitrogen percentages reported for FII+FIII by Hamaker et al (1995) to corresponding percentages reported in other works ranged between 0.955 and 1.062 with a mean of 1.003 ± 0.041. This value warranted the legitimacy of comparison (Table I). The contents of ASN appeared to be, at best, equal to the contents corresponding to FII+FIII+FIV. This was confirmed by considering the ratio of ASN to the content of FII+FIII+FIV, which ranges from 0.892 to 1.035 with a mean of 0.953 ± 0.056. This departure suggested that some proteins belonging to FIV would not be isolated in the ethanolic phase when Wallace's procedure is used. Sequential extraction of proteins present in FIV with salt and 2ME buffered first at pH 3 then at pH 10 revealed the occurrence of two subfractions (Landry and Moureaux 1981). The first has an amino acid composition similar to that of water-soluble, alcohol-soluble reduced glutelins and corresponds to 27 kDa γ-zein. The second has an amino acid composition similar to that of a subfraction extracted with salt and 2ME from protein bodies and containing a 58-kDa polypeptide rich in lysine (3%) (Vitale et al 1982, Ludevid et al 1985). Studies of the immunological cross-reactivity between proteins present in FII and FIII have suggested that proteins of 58 kDa in FIV, 28 kDa in FIV (27 kDa γ-zein), and 14 kDa in FIII (more probably 16 kDa γ-zein) would belong to the same family (Ludevid et al 1985). If so, protein of 58 kDa in FIV must be put together with zein proteins. It is noteworthy that a 58-kDa protein has been evidenced in the electrophoretic diagram of total zein by Lopez and Karkins (1991), but it was a dimer of 27 kDa γ-zein, devoid of lysine. Its presence, therefore, cannot explain the doubling of lysine content of total zein originating from quality protein maize with respect to that isolated from normal maize. On the other hand, the high value (1.035) of the ratio ASN/(FII+FIII+FIV) observed with W64A*o2* could be linked to the presence of high level of nonprotein in endosperm of *opaque 2* maizes (Landry and Moureaux 1980).

On this basis, a simple procedure for isolating total zein together with nonprotein nitrogen consists of subjecting defatted meal to three successive extractions with 70% (v/v) EtOH, 0.5% sodium acetate, and 0.6% 2ME. A fourth extraction with 0.5% acetate and 0.6% 2ME would allow 58-kDa protein of FIV to be

[1]Laboratoire de Chimie Biologique, INRA, INA PG. F-78850 Thiverval Grignon. Fax 033 130815373.

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TABLE I
Zein Content (%) of Whole Grain and Endosperm as Determined Using Sequential Extraction or Fractional Precipitation of Proteins

	Present Study and Others[a]			Hamaker et al (1995)		
Sample	**Genotypes (N%)[b]**	**FII+FIII[c]**	**FII+FIII+FIV[c]**	**ASN[d]**	**FI+FII[c]**	**Genotypes (N%)[b]**
Whole grain	(1)W64A (2.4)	53.4	61.1	56.2	51.0	W64A (2.39)
	(2)Tupesco (1.6)	44.5	56.5	50.4	46.5	B73 (1.67)
	(3)INRA260 (1.9)	49.5	59.5	59.7	52.5	Oh43 (2.02)
	(4)F2F7 (1.9)	50.4				
Endosperm			59.2			
	(1)W64A (1.6)	63.0	70.0[e]			
	(1)W64A (2.2)	66.1	71.3			
	(4)W64A	65.9	71.7	69.0	65.0	W64A (2.21)
	(5)W64A (1.6)	65.0	70.5			
	(6)W64A (2.1)	60.6	68.9			
	(7)Oh43	64.8	77.5	73.9	65.0	Oh43 (1.84)
	(3)INRA260 (1.6)	60.7	69.5	62.2	58.0	B73 (1.58)
	(5)W64A*o2* (1.5)	43.3	50.2			
	(6)W64A*o2* (1.8)	35.3	46.3	47.9	37.5	W64*o2* (1.84)

[a] (1) Present study; (2) Ortega et al (1986); (3) Landry and Moureaux (1980); (4) Gianazza et al (1976); (5) Miflin an Shewry (1977); (6) Païva et al (1991); (7) Misra et al (1972).
[b] Nitrogen % in sample, dry basis.
[c] Protein fractions FII, FIII, and FIV isolated by sequential extraction according to Landry and Moureaux (1970).
[d] Alcohol-soluble nitrogen (ASN) isolated by fractional precipitation according to Wallace et al (1990).
[e] FIV nitrogen extracted at acid then alkaline pH represents 5.8 and 1.2% of total nitrogen of endosperm.

isolated, if it must be included. Such a procedure leads total zein to be isolated in a volume lower than that required with the application of Wallace's method. Note that Wall et al (1988) extracted 69.8% of total nitrogen from W64A endosperm (2.45% nitrogen) free from salt-soluble nitrogen, using 70% (v/v) EtOH + 0.5% sodium acetate (twice) then 70% EtOH + 0.5% sodium acetate + 2% 2ME (twice).

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