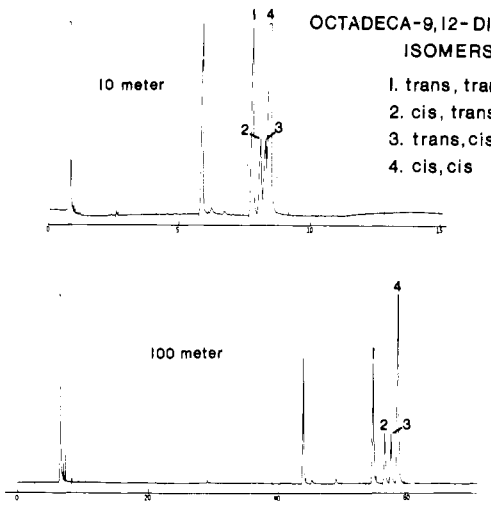

Figure 3. Methyl esters of the geometric isomers of 18:2ω6 chromatographed on 10-m and 100-m SP2340 glass capillary columns. The 10-m column was temperature programmed from 150 to 190 °C at 1.0 °C/min, and the 100-m column was temperature programmed from 150 to 200 °C at 0.4 °C/min.

100-m column completely separated the four geometric isomers of 18:2ω6cc, but the 10-m column did not completely separate the trans, cis, and cis,cis isomers.

CONCLUSION

Results were similar from the 10- and 100-m columns for the major fatty acids (greater than 1% normalized weight percent) and for the summation of individual fatty acids into various categories of interest to nutritionists, such as trans unsaturation, cis PUFA, and saturated fatty acids. The 10-m column could be useful for rapid analysis of large numbers of similar samples; however, the limitations of the 10-m as compared to the 100-m column for the separation and detection of minor fatty acids must be considered. The 2-m column was found to give rapid and reliable results for the analysis of standards or selective fatty acids, but it was inadequate for the quantitation of the complex mixtures of fatty acids found in most foods.

LITERATURE CITED

Ackman, R. G.; Eaton, C. A. *Fette, Seifen, Anstrichm.* **1978**, *80*, 21.
Allen, R. R. *J. Am. Oil Chem. Soc.* **1969**, *46*, 552.
Beare-Rogers, J. L. In "Geometric and Positional Fatty Acid Isomers"; Emken, E.; Dutton, H., Eds.; American Oil Chemists Society: Champaign, IL, 1979; pp 131-149.
Ettre, L. S.; March, E. W. *J. Chromatogr.* **1974**, *91*, 5.
Folch, J.; Lees, M.; Sloane Stanley, G. H. *J. Biol. Chem.* **1957**, *226*, 497.
Johansen, N. *Chromatogr. Newsl.* **1977**, *5*, 14.
Kaiser, R. *Fresenius Z. Anal. Chem.* **1961**, *189*, 1.
Metcalfe, L. D.; Schmitz, A. A.; Pelka, J. R. *Anal. Chem.* **1966**, *38*, 514.
Rooney, T. A.; Altmayer, L. H.; Freeman, R. R.; Zerenner, E. H. *Am. Lab. (Fairfield, Conn.)* **1978**, *10*, 81.
Slover, H. T.; Lanza, E. "Abstracts of Papers", 173rd National Meeting of the American Chemical Society, New Orleans, LA, March 1977; American Chemical Society: Washington, DC, 1977.
Slover, H. T.; Lanza, E. *J. Am. Oil Chem. Soc.* **1979**, *56*, 933.

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Distribution and Amino Acid Composition of Protein Groups Located in Different Histological Parts of Maize Grain

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From normal and *opaque-2* maizes (*Zea mays*), the whole grain and its histological parts, germ, endosperm, and envelopes (combined tip cap, pericap, and probably aleurone layer), were characterized by their dry matter and nitrogen content. For germ and endosperm, nonprotein nitrogen, albumins, globulins, zein, and G_1-, G_2-, and G_3-glutelins were isolated; for envelopes only the first three fractions were extracted. Various protein fractions of similar solubility exhibit only slight differences in amino acid composition. Proteins, on basis of location in grain, can be classified as follows: (1) endosperm-specific proteins, consisting of zein and G_1- and G_2-glutelins and found virtually in endosperm only; (2) basic proteins, making up all other fractions and occurring in all tissues. The influence of extraction conditions on the isolation of protein fractions is discussed.

The maize caryopsis, connected to the rachis (cob) by the tip cap, is a complex physiological entity containing various histological elements with different genetic origins and with specific functions. Thus, the outermost tissues, which consist of the pericap and the seed outer layers with the aleurone layer excluded, are genetically identical with the maternal plant and have a protective function. The endosperm, the hereditary makeup of which is two-thirds maternal and one-third paternal, is a tissue filled with storage material used by the growing embryo during germination. The germ, which has equal inheritance from both parents, is composed of the scutellum, a storage element, and of the embryonic axis, which is at the origin of the new plant. Consequently, the proteins present in these various histological elements must be differentiated

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by their quantity and quality alike.

The most extensive investigation aimed at the distribution of the Osborne protein groups according to their histological location in the maize grain was performed by Schneider et al. (1952). They showed that proteins extracted with 64% (w/w) aqueous ethanol and constituting zein are present in very low amounts in germ as well as in pericarp and may be treated as an endosperm-specific group as a first approximation. However, the very nature of some protein groups remains unknown in so far as on one hand nonprotein nitrogen is not differentiated from albumins and globulins and as on the other hand the glutelins are only identified through their extractibility and their insolubility in dilute sodium hydroxide. Indeed, in the case of the whole grain, alkali-soluble glutelins were shown to be a mixture of three subgroups isolated and termed G_1-, G_2-, and G_3-glutelins in an earlier study (Landry and Moureaux, 1970). Similar comments can be made concerning the studies of the nitrogen distribution in the endosperm and germ of maize grains carried out by Bressani and Mertz (1958) with the fractionation method developed by Mertz et al. (1958), and by Tsai (1979) with an extraction method close to that used by Schneider et al. (1952). At last, by collecting some results in the literature, in particular those reported by Christianson et al. (1965) and Boundy et al. (1967) related to nonprotein nitrogen, Wall and Paulis (1978) partially remedied the above remarks. Nevertheless, it has proved difficult to compare data between these researchers in view of the multiplicity of samples and experimental conditions. For example, in germ the ratios of salt-soluble nitrogen to total nitrogen of part were found to range from 57% (Paulis and Wall, 1969) to 63.5% (Schneider et al., 1952) or 82% (Mertz et al., 1958).

From a qualitative point of view, the amino acid compositions of total nitrogen of endosperm and germ were determined by Mertz et al. (1966) and that of pericarp by Boundy et al. (1967). The protein groups or subgroups, the composition of which was analyzed, are the following: in germ, albumins and globulins only (Paulis and Wall, 1969); in endosperm, albumins and globulins (Paulis and Wall, 1969; Sodek and Wilson, 1971), zeins and G_1-glutelins (Sodek and Wilson, 1971; Misra et al. 1975), and G_2- and G_3-glutelins (Misra et al., 1975) (when taking into account the most extensive investigations). But none of them is complete since for a given sample some protein subgroups have not been isolated and consequently not identified.

This paper deals with the distribution of several protein groups in germ, endosperm, and envelopes (this term was perferred to that of bran which refers to an industrial processing product) from the grain of normal and *opaque-2* (*o2*) maize. The protein fractions in germ and endosperm were obtained by selective extraction and their amino acid composition determined in the case of normal maize. The aim of this study was a more thorough characterization of the four glutelin (G_1, G_2, G_3, and insoluble) subgroups by the determination of their location in histological parts of grain. It is noteworthy that the classification of protein groups into basic and endosperm-specific proteins, as developed in this paper, has been already used in other studies to assess the quantitative variations in accumulation of protein fractions in the developing grain (Landry and Moureaux, 1976) as well as the kinetics of their degradation in endosperm during germination (Moureaux, 1979).

MATERIALS AND METHODS

The maizes studied were the crop generation of normal and *opaque-2* hybrids containing the same genetic background except for the *o2* gene. The samples were designated by I_1 and I_2 for INRA 260 of two different crops and by *o2* for *opaque-2*.

Wet grains, harvested at a 30% moisture level and stored at −10 °C (I_1 and *o2*) or at 5 °C (I_2) for some days, were divided manually into three parts, namely, envelopes, germ, and endosperm, by peeling off the outer integuments and dissecting out the germ with a scalpel. These three parts were freeze-dried and ground. The dry germ was first roughly ground and its oil removed with acetone at 10 °C before fine grinding. The meals were partially defatted at −10 °C by percolation on column with 5 mL of anhydrous acetone, followed by 2.5 mL of ethyl ether for 1 g of meal.

The nitrogen from defatted meal was extracted stepwise by a series of solvents according to the previously described procedure (Landry and Moureaux, 1970). Thus, 3.5-g samples were kept in suspension with 35 mL of extractant by magnetic stirring in 50-mL centrifuge tubes. The duration (minutes) and number of extractions with each solvent were as follows: step 1, 0.5 M NaCl, 60, 30, and 30 min; step 2, H_2O, 15 and 15 min; step 3, 55% isopropyl alcohol (w/w), 60, 30, and 15 min; step 4, 55% isopropyl alcohol (w/w) with 0.6% 2-mercaptoethanol (v/v), 30 and 30 min.; step 5, borate buffer, pH 10 (0.0125 M $Na_2B_4O_7$, 12 H_2O, and 0.02 M NaOH), with 0.6% 2-mercaptoethanol and 0.5 M NaCl, 60, 30, and 15 min; step 6, borate buffer, pH 10, with 0.6% 2-mercaptoethanol (v/v) and 0.5% sodium dodecyl sulfate ($NaDodSO_4$) (w/v), 60, 30, and 15 min. When albumins and globulins were extracted separately, the extraction conditions at step 1 were modified as follows: step 1a, H_2O, 60 and 30 min; step 1b, 0.5 M NaCl, 60 and 30 min. Extractions at steps 1 and 2 were performed at 4 °C and the others at room temperature. The solid material was isolated from extractants by centrifugation at 30000*g* for 15 min. For each solvent, supernatants were combined to give the extract. The extract obtained with water at step 2 contained minute amounts of nitrogen (<1% of total nitrogen of sample) and was discarded.

Salt-soluble proteins, albumins, and globulins were isolated respectively from the NaCl extract of step 1, water extract of step 1a, and NaCl extract of step 1b, after their precipitation consecutive to the addition of 50% trichloroacetic acid in these extracts to a final concentration of 10%. After being allowed to stand overnight, precipitates were isolated by centrifugation. The nitrogen of clarified supernatants (for extract 1) or of their mixture (for extracts 1a and 1b) represented nonprotein nitrogen.

Nitrogen was assayed with a micro-Kjeldahl method.

Amino acids analysis were carried out with a Phoenix Model K 8 000 automatic analyzer from proteins hydrolysates (24 h under reflux with constant-boiling HCl). No corrections were made for destruction of amino acids or incomplete hydrolysis.

RESULTS

In Table I are tabulated, for normal and *o2* maize grains, the amounts of dry matter and nitrogen per grain and its hand-dissected elements as well as the nitrogen content. The germ of the I_1 sample, when compared to that of the I_2 sample, has a weight 177% greater and a nitrogen content 20% lower. However, the I_1 and I_2 sample grains possess the same general characteristics as those reported by Earle et al. (1946) and by Schneider et al. (1952).

With respect to the germ of the *o2* grain, the slight increase, in absolute as well as relative value, of weight (except when the comparison is related to the I_1 sample) and of nitrogen amount is consistent with the more ex-

Table I. Dry Matter and Nitrogen Content of Whole Grain and Its Parts for Normal (I_1; I_2) and *opaque 2* (*o2*) Maizes[a]

	DM[b] in part, mg			DM part/DM grain, %			N[c] in part, mg			N part/DM part, %			N part/N grain, %		
	I_1	I_2	*o2*	I_1	I_2	*o2*	I_1	I_2	*o2*	I_1	I_2	*o2*	I_1	I_2	*o2*
germ	35	19.8	23	13.5	8.1	35	0.88	0.62	1.00	2.52	3.15	4.30	20.1	14.9	35.1
endosperm	208	205.0	112	80.0	84.0	61	3.34	3.36	1.73	1.60	1.64	1.55	76.5	80.5	60.7
envelopes	17	19.2	13	6.5	7.9	4	0.15	0.19	0.12	0.90	1.00	0.90	3.4	4.6	4.2
grain	260	244.0	148	100	100	100	4.37	4.17	2.85	1.71	1.68	1.92	100	100	100

[a] The values represent the average of a set of 200 cryodesiccated parts of 200 grains. [b] Dry matter. [c] Nitrogen.

Table II. Nitrogen Distribution in Whole Grain and Its Parts[a]

		% N									
		whole grain			endosperm			germ			envelopes
step	nitrogen fractions	I_1	I_2	*o2*	I_1	I_2	*o2*	I_1	I_2	*o2*	I_2
1[b]	salt-soluble N	22.4	(20.9)[c]	45.1	8.8	10.5	26.9	69.7	75.7	76.7	24.6
	nonprotein N	6.8	(8.0)	16.0	2.6	4.7	14.6	14.5	22.8	22.4	16.8
	salt-soluble P[d]	15.6	(12.9)	29.1	6.2	5.8	12.3	55.2	52.9	54.2	7.8
	albumins	nd[e]	(8.3)	nd	nd	2.7	nd	nd	40.2	nd	3.7
	globulins	nd	(4.6)	nd	nd	3.1	nd	nd	12.7	nd	4.1
3	zein	39.4	(41.8)	9.9	52.8	51.6	11.6	4.1	2.5	1.9	nd
4	G_1-glutelins	9.4	(5.8)	7.2	7.9	7.0	9.2	2.0	0.6	1.0	nd
	zein plus G_1	48.8	(47.6)	17.1	60.7	58.6	20.8	6.1	3.1	2.9	nd
5	G_2-glutelins	9.3	(9.2)	10.3	8.8	11.0	10.9	3.2	2.2	3.1	nd
	zein plus G_1 plus G_2	58.1	(56.8)	27.4	69.5	69.6	31.7	9.3	5.3	6.0	nd
6	G_3-glutelins	13.4	(14.8)	19.5	13.9	15.5	26.3	15.4	15.2	15.3	nd
	insoluble P	6.2	(7.5)	7.9	7.8	4.4	15.1	5.6	3.8	2.1	nd

[a] Expressed as percentage of nitrogen contained in the fraction to nitrogen contained in the part. [b] Numbers refer to the solvent used as described under Materials and Methods. [c] Values in parentheses are calculated from the data of Tables I and II. [d] Proteins. [e] Not determined.

tensive observations made by Bjarnason and Pollmer (1972) about the germ contribution to the protein quantity and quality of maize grain.

The proportions of nitrogenous fractions, expressed as a percentage of recovered total nitrogen, are given in Table II for two (I_1 and *o2* samples) or three (I_2 sample) parts of grain and for the whole grain. The protein group distribution for the whole grain from the I_2 sample was not determined, and the numbers in parentheses correspond to values as they may be deduced from the combination of data available in Tables I and II. The calculated values are seen to be virtually identical with the ones experimentally obtained from whole grains of the I_1 sample which have the same nitrogen level.

The different nitrogenous fractions appear to be unevenly distributed among different parts of the grain: the endosperm is typified by a low content of salt-soluble nitrogen and a high level of zein, whereas the reverse is recorded with the germ, where salt-soluble nitrogen and G_3-glutelins are the most prominent fractions. With regard to envelopes, water and NaCl extractions removed only 25% of the total nitrogen. The other proteins of this part were not submitted to selective extraction. However, it can be supposed, on the basis of amounts of hull proteins extracted in aqueous ethanol and alkali (Schneider et al., 1952), that proteins liable to be isolated at steps 3–5 do not exceed 20% of total nitrogen. Therefore, envelope proteins would mainly comprise G_3-glutelins and insoluble proteins.

The quantitative aspect of all these observations is summarized by the data in Table III. These data, deduced from values listed in Tables I and II, enhance the contribution of each nitrogen fraction, isolated from various histological parts, to the homologous fraction defined from the whole grain.

As seen from Table II, the *o2* gene has little or no effect on the distribution of protein groups occurring in the germ. These results support the identity of the overall amino acid composition of germs from normal and *o2* grains, as shown by Mertz et al. (1966). On the contrary, the introduction

Table III. Percentage of the Total Nitrogen of Each Nitrogenous Fraction Contained in Different Parts of Normal (I_2) Maize Grain

	%N		
fractions	germ	endosperm	envelopes
salt-soluble N[a]	54.6	40.6	4.8
NPN[b]	43.0	47.6	9.4
salt-soluble P[c]	61.9	36.3	1.8
albumins	72.9	25.9	1.2
globulins	41.8	55.1	3.1
zein	0.9	99.1	
G_1-glutelins	1.7	98.3	
G_2-glutelins	3.7	96.3	
zein plus G_1 plus G_2	1.4	98.6	
G_3-glutelins plus insoluble P[d]	12.9	71.9	15.2
salt-soluble P plus G_3 plus insoluble P	56.6	33.0	10.4

[a] Nitrogen. [b] Nonprotein nitrogen. [c] Proteins. [d] Values based on the assumption that envelope proteins not submitted to extraction only consist of G_3-glutelins and insoluble proteins.

of the *o2* gene is accompanied by marked alterations of the importance of protein groups of endosperm, as evidenced by many investigations (Sodek and Wilson, 1971; Misra et al., 1975; Gianazza et al., 1976) using a similar or identical extraction scheme. Thus, in *o2* endosperm, with respect to its normal counterpart, the levels of salt-soluble nitrogen, G_3-glutelins, and residual proteins are significantly higher whereas that of zein is lower. The comparison of these data with those obtained by Sodek and Wilson (1971) emphasizes the effect of environment or *o2* gene expression on the protein distribution in *o2* endosperm, since these authors reported that zein proportions made up 2.9 and 17.8% of total nitrogen in the two *o2* samples analyzed.

Amino acid compositions for separate protein groups in each histological part of I_2 grain are given in Table IV. For each solubility group no marked differences in composition are observed according to their localization to the extent that hydroxyproline, as detected in some pericarp proteins

(Boundy et al., 1967), is not taken into consideration.

The overall compositions of albumins and globulins from germ, endosperm, and envelopes are quite similar. However, germ albumins are slightly richer in all basic residues than their homologues of the endosperm. In addition, germ globulins are rich in histidine, arginine, and phenylalanine whereas those of the endosperm have a composition close to that of albumins of the same part, nevertheless with notable differences in histidine, aspartic acid (or asparagine), and proline.

The amino acid content of G_3-glutelins is independent of their location. The most obvious discrepancies involve arginine, proline, and glycine. On the contrary, the composition of insoluble proteins varies slightly with their location. The germ proteins have high content of arginine and glycine but are low in glutamic acid (or glutamine). The insoluble proteins from the endosperm are poor in lysine and rich in histidine and proline, whereas the nonextracted proteins from envelopes have an overall composition similar to that of unfractionated proteins of the pericarp (Boundy et al., 1967) and which is typified by a high level of leucine.

Prolamin and G_1- and G_2-glutelins extracted from germ share with the homologous groups isolated from the endosperm some features related to composition, namely, a high content in hydrophobic amino acids and a low level in lysine for prolamin and high proportion of methionine for G_1-glutelins and of histidine for G_2-glutelins. However, slight but significant differences suggest the presence of specific proteins.

DISCUSSION

Although the efficiency of hand dissection was not checked by observations with an optical microscope, it stands to reason that each of the three histological parts isolated in this work are relatively free of the other parts. However, it is worthy of note that envelopes, which, in the present study, refer to a mixture of pericarp and tip cap, exhibit a nitrogen content intermediate between that of a mixture of the same histological elements as calculated from the data recorded by Schneider et al. (1952) and that calculated by Hinton (1953). It is therefore probable that peeling induces a partial removal of the aleurone layer, the presence of which would result in a variability in the proportions of salt-soluble nitrogen of the pericarp.

On the other hand, the fractions extracted from germ at steps 3–5 can be looked upon as constituted by specific proteins or by some particular proteins strongly contaminated by zein and G_1- and G_2-glutelins originating from the endosperm. A circumstantial evidence for the first assumption is provided by the data reported by Schneider et al. (1952), who found in germ, carefully isolated, some alcohol- and alkali-soluble proteins in proportions similar to those listed in Table II for steps 3–5. Other evidence for specific alcohol-soluble protein in germ is indicated by the observations of Tsai (1979). Indeed, this worker proved that 10% of total nitrogen of a germ preparation, checked as free of endosperm contamination, could be extracted at 60 °C by 70% ethanol containing 1 mM 2-mercaptoethanol and that most of electrophoretic components of these alcohol-soluble proteins had an amino acid composition similar, but not identical, to homologous components isolated from endosperm. Therefore, the contaminations, if any, between parts are very limited and the results of this work must give a true picture of protein spectra of parts.

As pointed out in Table III, nearly all the grain proteins extracted at first by aqueous isopropyl alcohol without and with reducing agent and then by pH 10 buffer with re-

Table IV. Amino Acid Compositions of Protein Groups Isolated from Different Parts of Normal (I_2) Maize Grain[a]

step	protein group	part	composition, no. of residues/1000 residues														
			Lys	His	Arg	Asx	Thr	Ser	Glx	Pro	Gly	Ala	Cys	Val	Met	Ile	Leu	Tyr	Phe
1a	albumins	germ	70	23	64	102	52	63	125	42	97	95	(18)[b]	67	(17)[b]	37	73	24	32
		endosperm	57	18	50	101	61	66	111	65	99	108	nd[c]	72	(11)	42	82	25	32
		envelopes	63	17	41	105	54	72	106	59	103	109	nd	74	(13)	38	81	26	38
1b	globulins	germ	54	34	107	86	42	89	114	49	110	91	nd	63	(5)	31	53	24	46
		endosperm	52	24	52	96	51	66	128	79	101	99	nd	61	(14)	35	77	29	36
		envelopes	69	25	60	104	56	64	110	62	114	105	nd	59	nd	39	73	24	37
	salt-soluble proteins[d]	germ	63	25	71	90	50	71	134	48	101	99	nd	64	(14)	35	72	23	40
		endosperm	57	23	54	89	56	65	121	70	101	111	nd	67	(5)	38	82	22	39
		envelopes	69	22	48	102	59	67	107	52	108	112	nd	66	(13)	38	77	24	36
6	G_3-glutelins	germ	55	24	58	82	53	64	107	44	108	107	(7)	73	(9)	44	99	26	40
		endosperm	58	23	47	86	50	62	112	59	86	108	(14)	72	(12)	46	97	26	42
	insoluble P[e]	germ	59	31	57	73	57	72	89	72	156	105	(17)	54	(16)	24	74	26	26
		endosperm	32	40	39	56	48	54	124	130	104	80	nd	70	(19)	31	89	22	26
3	G_3-glutelins plus insoluble P	envelopes	55	23	34	89	69	74	135	88	108	117	nd	60	(9)	39	113	24	41
	zein	germ	5	11	15	60	33	73	206	105	38	142	(3)	39	(9)	25	165	32	39
		endosperm	1	9	10	54	30	69	220	97	21	138	(3)	36	(10)	29	187	35	51
4	G_1-glutelins	germ	9	14	22	44	42	75	196	113	97	118	nd	35	(32)	16	115	42	30
		endosperm	1	12	17	34	37	63	198	109	58	125	(7)	38	(51)	29	141	48	32
5	G_2-glutelins	germ	48	49	68	77	45	73	152	92	124	77	nd	56	(9)	24	61	15	29
		endosperm	9	62	25	21	56	58	210	205	83	62	nd	59	(6)	20	94	17	13

[a] Amino acids expressed as number of residues per 1000 residues (single analysis). [b] Values in parentheses are estimations. [c] Not determined. [d] Composition determined from a mixture of aliquots of extracts 1a and 1b. [e] Proteins.

ducing agent appeared to originate from the endosperm. On this basis zein and G_1- and G_2-glutelins can be assumed to be endosperm-specific proteins, unlike salt-soluble proteins, G_3-glutelins, and residual proteins which, in spite of their uneven distribution within grain, are scattered in all tissues. They may be looked upon as basic proteins. These consist chiefly, by neglecting the low amounts of storage proteins, represented by globulins of scutellum and aleurone layer, of proteins, functional or structural, which play a role, other than that of reserve, in the cell metabolism during grain development and which can be found in all vegetative plant structures. Such a discrimination, that is also valid for the *o2* sample, has proved to present an apparent usefulness in the study of the accumulation of protein groups in the development of their grain (Landry and Moureaux, 1976) as well as that of their breakdown in the endosperm of the germinating grain (Moureaux, 1979). Thus, all the basic proteins are present in the very immature grain before the synthesis of endosperm-specific proteins, and at the beginning of maturity the disappearance of some salt-soluble proteins is correlated with their aggregation into G_3-glutelins. During germination the opposite phenomenon, namely, disaggregation of G_3-glutelins into salt-soluble proteins, takes place in the endosperm before the hydrolysis of all the protein groups.

Plant proteins can be classified according to several ways, as recently reviewed by Boulter and Derbyshire (1977). In the present work, the distribution of maize caryopsis proteins into two classes is close to that outlined by Yemm (1958), who distinguishes reserve and protoplasmic proteins. The former are storage products located in endosperm or cotyledons; the latter are "intimately associated with the structure and function of cells". According to this author, in barley caryopsis, albumins and globulins, on account of their low amounts and of their amino acid composition, are regarded as protoplasmic proteins; prolamins and glutelins are regarded as reserve proteins. The distinction of these last two groups has been kept in view of differences of their behavior in developing as well as germinating grain. As can be seen from this investigation and others (Moureaux and Landry, 1972a, b; Misra et al., 1975), the isolation of three glutelin subgroups, and of G_3 in particular, affords a further insight into biochemical properties of proteins of this group and allows one to establish a good correlation about the classifications of proteins based on their extractibility or their location or function in grains. Nevertheless, the characterization of the proteins present in subcellular organelles would allow more accurate information to be obtained about their function in cell physiology. In particular, it would be interesting to know if G_2-glutelins are stored in protein bodies which contain zein and probably G_1-glutelins.

The classification of protein groups into basic and endosperm-specific proteins is different from that drawn up earlier (Landry and Moureaux, 1970). Indeed, G_2-glutelins, because of their extractability in aqueous buffer and of the high proportion of their basic residues, are put together with "basic proteins" under the name of water-soluble proteins. But some data provide evidence that G_2-glutelins can also be allied to zein and G_1-glutelins. Indeed, Paulis and Wall (1977) resorted to 70% ethanol containing 0.5% sodium acetate and 0.1 M 2-mercaptoethanol to extract alcohol-soluble reduced glutelins from maize endosperm meal deprived of salt-soluble proteins and zein. Upon dialysis of extract against water, these glutelins separated into water-soluble and water-insoluble fractions, which can be identified as G_2- and G_1-glutelins, respectively, on the basis of electrophoretic data, when compared to these of Misra et al. (1976), and of amino acid composition. Therefore, G_2-glutelins are amenable to extraction with aqueous alcohols, provided that the extractants contain a reducing agent and a small amount of salt such as sodium acetate. Moreover G_2-glutelins, like zein and G_1-glutelins, are rich in proline and poor in lysine. Consequently, the existence of two classifications only emphasizes the amphiphilic nature of G_2-glutelins.

The similarity of some physicochemical properties between the protein groups or subgroups of a same class hints at the possibility of their partial coextraction as a consequence of peculiar operational conditions. Thus, for the endosperm of W 64 A variety having the same nitrogen content as that of INRA 260 variety, Soave et al. (1977) reported the following protein distribution (expressed as percent of total nitrogen): salt-soluble nitrogen, 6.5; zein, 46; G_1-glutelins, 19; G_2-glutelins, 5.5; G_3-glutelins, 9.7; insoluble, 13.1. In fact, the wide discrepancies between these values and those listed in Table II disappear when the diverse fractions are grouped into basic nitrogen (basic protein plus nonprotein nitrogen) and endosperm-specific proteins. Indeed, the former class amounts to 29.3% (Soave et al., 1977) or 30.4% (Table II) and the latter one to 70.5% or 69.6%. The near identity of data suggests that the vigourous stirrings, as used by these authors, bring about a partial insolubilization of salt-soluble proteins and G_3-glutelins. The same holds for zein, a part of which is only solubilized in the presence of reducing agent. Moreover, an incomplete removal of NaCl from meal would be at the origin of a partial extraction of G_2-glutelins with G_1-glutelins. It would be the same for zein-2, isolated by Sodek and Wilson (1971) with 55% isopropyl alcohol and 0.6% 2-mercaptoethanol from nondelipidated endosperm of WF9 × M14 and R892 × R75 maizes, since this fraction possesses an amino acid composition similar to that of a mixture of G_1- and G_2-glutelins. Therefore, the experimental conditions as specified in this work seem to prove the most satisfactory for assessing the distribution of protein groups in maize grains. Also, it is worth remembering that the isopropyl alcohol concentration was chosen as extracting, according to Swallen (1941), the maximum amount of proteins from maize gluten meal at room temperature.

With regard to albumins and globulins, the examination of their amino acid composition, as reported in this work and in the studies made by Paulis and Wall (1969) and by Sodek and Wilson (1971), also shows the influence of isolation methods on the quality of both subgroups. Thus, for germ albumins and globulins, only small differences are detected between our data and those reported by Paulis and Wall (1969). Likewise for endosperm albumins, all compositions reported are similar. But if endosperm globulins obtained by us or by Sodek and Wilson (1971) are similar and close to endosperm albumins, those prepared by Paulis and Wall (1969) are near to germ globulins. Against the above background, it would appear that the use of water, as the first extractant of endosperm proteins [this study and that of Sodek and Wilson (1971)] is liable to lead to the insolubilization of some albumins which are later extracted with NaCl solution and of some globulins which would pass over into glutelins extractable (G_2 and perhaps G_3) or not.

At last, it appears from Table III that zein, when isolated from an alcoholic extract originating from the whole grain, contains some germ prolamins. This contamination is very low and probably of the same importance as that resulting from the presence of endosperm protein species, which are

allied to G_1- or G_2-glutelins and coextracted with zein. It is the reason why in an elaborate study on zein (Landry and Sallantin, 1978; Landry, 1979) the whole maize grain, rather than the endosperm, was used as the starting material, thus eliminating the disadvantages inherent to hand dissection of grain.

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LITERATURE CITED

Bjarnason, M.; Pollmer, W. G. *Z. Pflanzenzuechtg.* **1972**, *68*, 83–89.
Boulter, D.; Derbyshire, E. In "Plant Proteins"; Norton, G., Ed.; Butterworths: London and Boston, 1977; pp 1–24.
Boundy, J. A.; Wall, J. S.; Turner, J. E.; Woychik, J. H.; Dimler, R. J. *J. Biol. Chem.* **1967**, *242*, 2410–2415.
Bressani, R.; Mertz, E. T. *Cereal Chem.* **1958**, *35*, 227–235.
Christianson, D. D.; Wall, J. S.; Cavins, J. F. *J. Agric. Food Chem.* **1965**, *13*, 272–276.
Earle, F. R.; Curtis, J. J.; Hubbard, J. E. *Cereal Chem.* **1946**, *23*, 504–510.
Gianazza, E.; Righetti, P. G.; Pioli, F.; Galante, E.; Soave, C. *Maydica* **1976**, *21*, 1–17.
Hinton, J. J. C. *Cereal Chem.* **1953**, *30*, 441–445.
Landry, J. *Biochimie* **1979**, *61*, 549–558.
Landry, J.; Moureaux, T. *Bull. Soc. Chim. Biol.* **1970**, *52*, 1021–1037.
Landry, J.; Moureaux, T. *Qual. Plant.—Plant Foods Hum. Nutr.* **1976**, *25*, 343–360.
Landry, J.; Sallantin, M. 6th International Cereal and Bread Congress, Winnipeg, Canada, 1978; Abstract B 3-4, 16-22.9.
Mertz, E. T.; Llyod, N. E.; Bressani, R. *Cereal Chem.* **1958**, *35*, 146–155.
Mertz, E. T.; Nelson, O. E.; Bates, L. S.; Veiron, O. H. *Adv. Chem. Ser.* **1966**, *No. 57*, 228–242.
Misra, P. S.; Mertz, E. T.; Glover, D. V. In "High Quality Protein Maize"; CIMMYT, Purdue University, Ed.; Dowden, Hutchinson and Ross: Stroudsburg, PA, 1975; pp 291–305.
Misra, P. S.; Mertz, E. T.; Glover, D. V. *Cereal Chem.* **1976**, *53*, 705–711.
Moureaux, T. *Phytochemistry* **1979**, *18*, 1113–1117.
Moureaux, T.; Landry, J. *Physiol. Vég.* **1972a**, *10*, 1–18.
Moureaux, T.; Landry, J. *C. R. Hebd. Seances Acad. Sci., Ser. D* **1972b**, *265*, 3309–3312.
Paulis, J. W.; Wall, J. S. *Cereal Chem.* **1969**, *46*, 263–273.
Paulis, J. W.; Wall, J. S. *Cereal Chem.* **1977**, *54*, 1223–1228.
Schneider, E. O.; Earley, E. B.; DeTurk, E. E. *Agron. J.* **1952**, *44*, 161–169.
Soave, C.; Viotti, A.; Salamini, F.; Gentinetta, E.; Gianazza, E.; Righetti, P. G. In "Techniques for Separation of Barley and Maize Proteins"; Miflin, B. J.; Shewry, P. R., Eds.; CEC: Luxembourg, 1977; pp 61–68.
Sodek, L.; Wilson, C. M. *J. Agric. Food Chem.* **1971**, *19*, 1144–1150.
Swallen, L. C. *Ind. Eng. Chem., Ind. Ed.* **1941**, *33*, 394–398.
Tsai, C. Y. *Biochem. Genet.* **1979**, *17*, 1109–1119.
Wall, J. S.; Paulis, J. W. *Adv. Cereal Sci. Technol.* **1978**, *2*, 135–219.
Yemm, E. W. In "Encyclopedia of Plant Physiology, Vol. VIII: Nitrogen Metabolism"; Ruhland, W., Ed.; Springer-Verlag: Berlin, 1958; pp 315–332.

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Separation of Phenolics of *Sorghum bicolor* (L.) Moench Grain

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Two column supports, useful in the resolution of sorghum grain phenolics, are introduced. The first, Sepharose CL-6B, divides the phenolics into three main groups, F_1, F_2, and F_3, while the second, Sephacryl S-200, subdivides the three fractions obtained from the Sepharose column. A continuous automated monitoring system is described for polyphenol detection. This system can utilize either Folin–Ciocalteu reagent or a solution of $FeCl_3$ in Rochelle salt. On addition of alkali, the Folin detection system monitors total phenolic hydroxyls present, while the $FeCl_3$ method monitors only those hydroxyl phenolics which are spatially acceptable for complexing with the Fe^{3+}. The F_3 group phenolics are the largest in size, consisting of two polymeric forms, one of molecular weight approximately 10 000 and the other of molecular weight greater than 80 000.

Work has been done on the separation and nature of the phenolics of sorghum. Blessin et al. (1963) isolated a material after extracting grain with water, by diphasic solvent distribution and separation on a Dowex 1-X10 column. They tentatively noted that cold acid treatment yielded fisetinidin, while hot acid caused oxidation to a flavonol. Later, Yasumatsu et al. (1965) extracted sorghum with methanol and using a crystalline cellulose column separated the extract into three leucoanthocyanidins. Boiling acid hydrolysis yielded eriodictyol and pelargonidin, though it is probable that the eriodictyol arose by oxidation from luteoferol (leucoluteolinidin).

Bate-Smith (1969) prepared luteoferol from eriodictyol by $NaBH_4$ reduction and showed its presence in both birdproof (high tannin content) and nonbirdproof sorghum cultivars. However, he did not attempt any separatory work and based his conclusions on color changes peculiar to luteoferol.

Nip and Burns (1969, 1971) studied both red and white cultivars of sorghum (colors may have nothing to do with tannin content). They extracted grain with acidified methanol and purified the extract by paper chromatography. Identification by comparison with two known compounds (not authenticated samples) suggested the

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