Table II. Peak Heights of Different Procyanidin Polymers Isolated by HPLC[a]

stage of maturity	peak heights, mm, for peak no.[b] 1	2	3	4	5	6	7
preflower	>215	0	0	0	0	0	0
midflower	215	0	0	0	0	0	0
late flower	192	0	0	0	0	0	0
milk	163	54	42	51	44	42	0
soft dough	180	46	54	71	68	66	63
hard dough	35	33	39	52	54	49	52
physiologically mature	81	0	35	35	30	48	58
time of harvest	79	0	0	0	41	54	53

[a] 50 μL was injected from a 10 mg of ground grain/mL of 70% acetone extract. [b] 1 = catechin; 2 = dimer; 3 = trimer a; 4 = trimer b; 5 = unknown a; 6 = unknown b; 7 = unknown c.

stage which contained the full complement of low molecular weight procyanidins. The more immature stages did not contain all the peaks shown here, and in the more mature stages the peaks of the lower molecular weight compounds began to diminish and disappear. Two properties were observed which seemed to be characteristic of procyanidins when run on HPLC. The pen did not return to base line between peaks, and this appeared to be independent of the solvents and column packings used. Also, the peaks became broader as the molecular weights of the procyanidins increased. The peak broadness could be due to a mixture of isomers eluting together as one peak; i.e., the chromatographic system failed to separate them.

The patterns of the procyanidins for all developmental stages were incorporated into Table II. The peak heights are reported, and they show the development of the low molecular weight procyanidins in the immature sorghum grain. Catechin was the only procyanidin compound present during the flowering stage. Only after fertilization occurred and the inner integument developed into a testa did the rate of procyanidin concentration increase. During the milk, soft dough, and hard dough stages, the catechin content decreased. Presumably this represents a period of rapid procyanidin synthesis when the catechin was being incorporated as the terminal unit of the polymer.

The bulk of procyanidin synthesis must have occurred during this period as in later stages of grain development neither the dimer nor trimer forms could be found. The mature grain contained only the high molecular weight forms of procyanidin. This pattern agrees with other work (Glennie, 1981) where extractable procyanidins were measured and found to reach a maximum at the hard dough stage. The HPLC patterns are consistent with the idea that procyanidins develop sequentially from a monomer through dimer and trimers, until they reach high molecular weight forms which are probably insoluble.

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Rye Prolamins: Extractability, Separation, and Characterization

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Compared with some concentrations of aqueous 1-propanol and 2-propanol, 60% (w/w) ethanol was found to be the best extractant of rye prolamins (secalins) with respect to purity. Rye meal defatting with water-saturated 1-butanol before extraction resulted in a 40% loss of secalin yield. Secalins were separated by ion-exchange chromatography and gel filtration. They are distributed in three groups named A, B, and C by order of increasing molecular weight (M_r). Like ω-gliadins and C hordeins, C secalins (M_r 38 000) have high Glx and Pro contents and are free of sulfur amino acids. B secalins (M_r 29 000) are similar to α-, β-, and γ-gliadins and therefore are probably the only secalins noxious for gluten-sensitive people. A secalins (M_r 16 000) have molecular weights in the same range as low molecular weight gliadin and A hordein molecular weight. They are Lys and His free and have much higher sulfur amino acid and Tyr contents than other secalins. Leu, Asx, and Arg are the N-terminal amino acids of A, B, and C secalins, respectively.

Like wheat, rye can be used for bread making though its baking quality is clearly lower than that of wheat. Moreover, rye is noxious for gluten-sensitive people although to a lesser extent than wheat (Dicke et al., 1953; Anand et al., 1978; Kasarda, 1978). Therefore rye is an interesting material for the study of relations between protein composition and functional properties on the one hand and for the study of relations between protein com-

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position and toxicity on the other hand.

Most of the works concerning rye endosperm proteins have been recently summarized by Simmonds and Campbell (1976). Rye alcohol-soluble proteins have received special attention: they have been analyzed by gel filtration (Preston and Woodbury, 1975), and their electrophoretic patterns at pH 3.2 have been shown to be specific of each variety (Alexandrescu et al., 1977).

In this paper, we report results concerning rye prolamins (secalins) that we define as the rye endosperm proteins, soluble in water–alcohol mixutres free of reducing agent, which give bands in the same region as wheat gliadins in gel electrophoresis at pH 3.2.

Our results concern the extractability of rye secalins by different alcohols and the separation of these proteins by ion-exchange chromatography on sulfopropyl-Sephadex C-50 and gel filtration on Sephadex G-100. Molecular weights, amino acid compositions, and N-terminal amino acids of the fractions isolated by this method have been determined.

MATERIALS AND METHODS

Extractability of Rye Proteins by Alcohols. Rye (*Scale cereale* cv. Beaulieu) was milled experimentally to 53% extraction. The nitrogen content of the flour used for protein extraction was 1.6% (dry basis) and its water content 13%.

All extractions were carried out by magnetic stirring of 1 g of flour in 10 mL of solvent at 20 °C. Flour was twice defatted with water-saturated 1-butanol (60 and 30 min) and then extracted with 0.5 M NaCl (60 min) and water (20 min) before alcohol extractions (60 min). The range of concentrations of the three alcohols tested, 1-propanol (*n*-PrOH), 2-propanol (*i*-PrOH), and ethanol (EtOH), was 10–80% (w/w). After being magnetically stirred all suspensions were centrifuged at 7700*g* for 10 min at 20 °C. Nitrogen contents of the supernatants were determined by the Kjeldahl method.

Analysis of Alcohol Extracts by Gel Filtration. The compositions of the proteins extracted by 55% *i*-PrOH and 60% EtOH were compared by chromatography on Sephacryl S200. These proteins were prepared by direct extraction of 50 g of flour with 500 mL of each alcohol after the flour had been twice defatted with 500 mL of water-saturated 1-butanol. After centrifugation at 1000*g* (20 min), EtOH and *i*-PrOH supernatants were diluted 3 and 5 times, respectively, with 1.5 and 1.25% NaCl solutions. After 48 h at 4 °C, suspensions were centrifuged at 15000*g* for 20 min at 4 °C. Precipitates of crude secalin were dried under vacuum over H_2SO_4.

Sephacryl S200 columns (4 × 110 cm) were equilibrated with the solvent AUC [0.01 M acetic acid; 3 M urea; 0.01 M cetyltrimethylammonium bromide (Meredith and Wren, 1966)] and loaded with 100 mg of protein dissolved in 5 mL of solvent. The elution flow was 17 mL/h and 7-mL fractions were collected. The fractions corresponding to one peak of the elution curve were pooled and dialyzed against 0.05 M CH_3COOH. The proportions of the four fractions with respect to the recovered material were calculated on the basis of their weight after freeze drying. The proportions were used to calculate the weight of each fraction contained in the different precipitates (Table I).

Ion-Exchange Chromatography on Sulfopropyl-Sephadex C-50 (SPS). The procedure was very similar to that used for the isolation of gliadin fractions (Charbonnier and Mossé, 1980). The column (5 × 80 cm) was packed with SP Sephadex C-50 gel equilibrated with a pH 3.1 buffer (2 M urea; 0.04 M ethylenediamine; 0.08 M HCl; CH_3COOH) and was loaded with 1 g of crude secalin dissolved in 40 mL of buffer. The column was successively eluted with 2700 mL of the pH 3.1 buffer and a 0.5 M CH_3COONa solution in the same buffer without CH_3COOH at a flow rate of 45 mL/h. Fractions (22.5 mL) were collected.

Gel Filtration of SPS Fractions on Sephadex G-100. When the starch gel diagram of a SPS fraction showed constituents which had been found in different S200 fractions, this SPS fraction was rechromatographed on Sephadex G-100 columns (2.5 × 107 cm) equilibrated with 0.1 M CH_3COOH. Columns were loaded with 70–100 mg of SPS fraction dissolved in 4–5 mL of 2 M urea–CH_3COOH pH 3.1 buffer and eluted at 12 mL/h. Fractions (6 mL) were collected.

Characterization. Starch gel electrophoreses at pH 3.2 were carried out according to conditions previously described (Landry et al., 1965).

For amino acid analysis, 5-mg protein samples were hydrolyzed under vacuum in 6 N HCl at 110 °C for 24 and 48 h. However, hydrolyses of low molecular weight secalins were carried out with 2-mg protein samples only because of the small quantities of available material. Sulfur amino acids were determined after performic acid oxidation. Tryptophan was assayed by the method of Slump and Schreuder (1969).

N-Terminal amino acids were determined by the dansyl chloride procedure (Narita et al., 1975) and molecular weights by polyacrylamide gradient gel electrophoresis according to conditions described in the Pharmacia Fine Chemicals handbook "Molecular weight determination using electrophoresis". The electrophoresis buffer was 0.04 M Tris, 0.02 M sodium acetate, and 2 mM EDTA, pH 7.4, containing 0.2% $NaDodSO_4$. Sample buffer was 10 mM Tris-HCl, pH 8.0, and 1 mM EDTA, containing 2.5% $NaDodSO_4$ and 5% β-mercaptoethanol. Pharmacia PAA 4/30 gels were calibrated with the Pharmacia "low molecular weight calibration kit" composed of phosphorylase *b* (M_r 94000), albumin (M_r 67000), ovalbumin (M_r 43000), carbonic anhydrase (M_r 30000), trypsin inhibitor (M_r 20100), and α-lactalbumin (M_r 14400).

RESULTS AND DISCUSSION

Rye Prolamin Extractability. Nitrogen dissolved in NaCl and water made up 40% of the total nitrogen of the rye flour, whereas only 20% of Cappelle wheat flour nitrogen was dissolved under the same conditions. This difference between rye and wheat has already been pointed out by Chen and Bushuk (1970) and Preston and Woodbury (1975).

The proportions of nitrogen solubilized in alcohol solutions are presented in Figure 1. A maximum of 12.8% of total nitrogen was extracted by 60% EtOH whereas nearly 18% was extracted by 30% *n*-PrOH and 40% *i*-PrOH. The most efficient concentrations of *n*-PrOH and *i*-PrOH for the extraction of rye alcohol-soluble proteins are very similar to those found by Laurière et al. (1976) for the extraction of barley alcohol-soluble proteins (35% *n*-PrOH and 40% *i*-PrOH). However, the highest extractability of barley alcohol-soluble proteins with EtOH was found at a concentration of 45% which is clearly lower than that found for rye.

When crude secalins were precipitated from a 55% *i*-PrOH extract, the weight of precipitate was smaller than the weight of the precipitate obtained from the 60% EtOH extract (Table I) because the solubility of rye alcohol-soluble proteins in 11% *i*-PrOH (5 times diluted 55% *i*-PrOH) was clearly higher than the solubility of these proteins in 20% EtOH (3 times diluted 60% EtOH) (Figure 1).

Table I. Weights and Compositions of Precipitates Obtained from Rye Flour Alcoholic Extracts

extraction conditions	precipitates,[a] mg	proportions and weights of S200 fractions							
		1		2		3		4	
		%	mg	%	mg	%	mg	%	mg
60% EtOH, defatting	743	21.7	161.2	33.5	249	42.7	317.2	2.1	15.6
60% EtOH, no defatting	1555.4	40.1	623.7	26.9	418.4	33	513.3		
55% *i*-PrOH, defatting	603.3	62.7	378.2	9.1	54.9	28.2	170.1		

[a] Obtained from 50 g of flour.

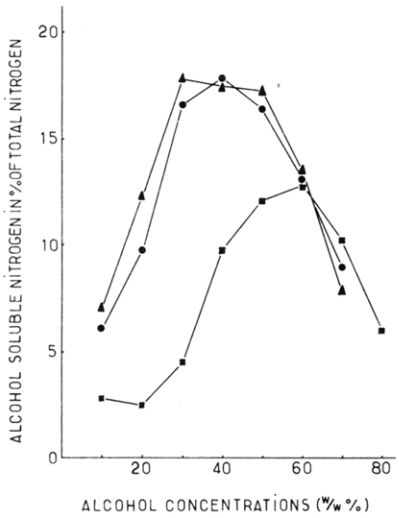

Figure 1. Extractability of alcohol-soluble proteins in aqueous solutions of *n*-PrOH (▲), *i*-PrOH (●), and EtOH (■) as a function of alcohol concentrations.

i-PrOH-soluble proteins were extracted with 55% *i*-PrOH, because the precipitate obtained from a 40% *i*-PrOH extract was only partially soluble in the AUC solvent used for gel filtration on Sephacryl S200.

The fractionation of 60% EtOH-soluble rye proteins by chromatography on Sephacryl S200 and the electrophoretic diagrams of the fractions are presented in Figure 2. Fraction 1 showed trailings and some constituents migrating in the same region as constituents of fraction 2. Proteins of fraction 2 migrated in the region of fast ω-gliadins. They were named C secalins whereas proteins of fraction 3 which migrated in the region of slow β-gliadins were named B secalins. The slower moving proteins of fraction 4, with mobilities similar to those of α-gliadins, were named A secalins, whereas the fast-moving constituents of this fraction were considered as albumins on the basis of their fast migration in electrophoresis at acid pH and of their amino acid composition (see below).

Undefatted flour extracted with 60% EtOH gave a higher proportion of high molecular weight proteins (fraction 1) than defatted flour. This proportion was higher again in the 55% *i*-PrOH extract (Table I), whereas starch gel diagrams of 60% EtOH and 55% *i*-PrOH precipitates isolated from defatted flour and the 60% EtOH precipitate isolated from undefatted flour do not show any clear difference (Figure 3).

As proteins eluted in the first fraction give trailings in starch gel at pH 3.2 and only one 54 000 band, after reduction, in polyacrylamide gradient gel electrophoresis (see below), they were considered as low molecular weight glutelins because of the similarity of their electrophoretic behavior with the low molecular weight alcohol-soluble glutenins of wheat. In a defatted flour these alcohol-soluble glutelins have more affinity for *i*-PrOH than for EtOH. On the other hand, their affinity for EtOH is considerably lessened by flour defatting (Table I). This

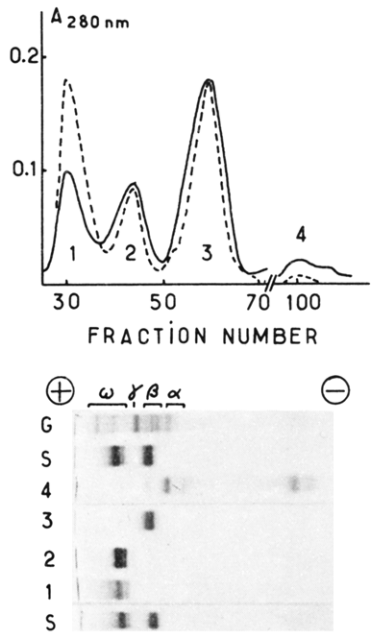

Figure 2. Starch gel electrophoresis at pH 3.2 (bottom) of fractions isolated by chromatography of crude secalin on Sephacryl S200 (top). (—) 60% EtOH extract from defatted flour. (- - -) 60% EtOH extract from nondefatted flour. S = crude secalin extracted from defatted flour with 60% EtOH. G = crude Cappelle wheat gliadin; 1, 2, 3, and 4 = S200 fractions.

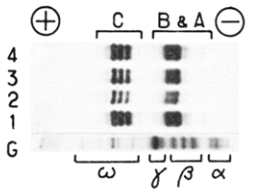

Figure 3. Starch gel electrophoresis at pH 3.2 of crude secalin precipitates. 1 and 4 = 60% EtOH extract from defatted flour. 2 = 60% EtOH extract from undefatted flour. 3 = 55% *i*-PrOH extract from defatted flour. G = crude Cappelle gliadin.

may result from splitting of secalin–glutelin bonds in which lipids would be involved or from a decrease of polarity resulting from the removal of polar lipids bound to these glutelins.

The proportion of alcohol-soluble glutelins is therefore smaller in precipitate of crude secalin extracted from defatted flour with 60% EtOH than in crude secalins extracted from undefatted flour with 60% EtOH or from defatted flour with 55% *i*-PrOH. However, the use of water-saturated *n*-BuOH as defatting solvent causes protein denaturation (Morton, 1955) which contributes to a decrease of yield of precipitate higher than 50% when extraction is carried out with 60% EtOH (Table I). A loss of yield of gliadin resulting from a wheat flour defatting with water-saturated *n*-BuOH has also been pointed out by Mecham and Mohammad (1955). However, defatting did not significantly change the proportions of fractions

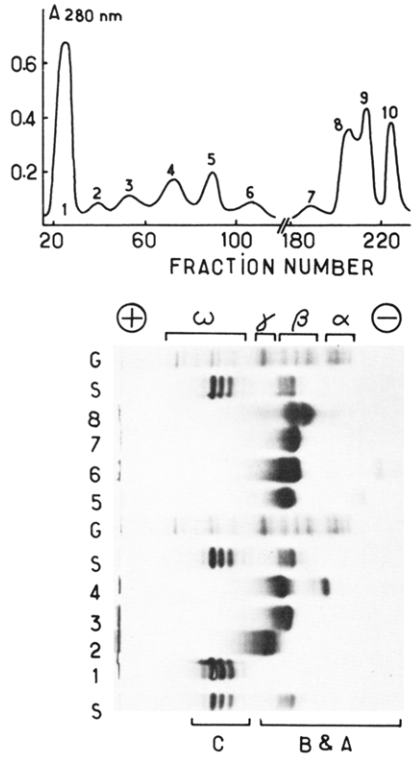

Figure 4. Starch gel electrophoresis at pH 3.2 (bottom) of fractions isolated by chromatography of crude 60% EtOH soluble secalin on sulfopropyl-Sephadex C-50 (top). S = crude secalin. G = crude gliadin. 1–8 = SPS fractions.

2 and 3 with respect to the sum of these fractions: $2/(2 + 3) \sim 45\%$ and $3/(2 + 3) \sim 55\%$ in both defatted and undefatted flour extracts. These proportions were quite different in the *i*-PrOH extract which is characterized by a particularly low content of C secalins.

Sixty percent EtOH is therefore the most specific solvent for the extraction of rye endosperm alcohol-soluble proteins that we defined as secalins.

Separation of Secalins. The fractionation of 60% EtOH soluble crude secalin by chromatography on SP Sephadex and the electrophoretic diagrams of the isolated fractions are shown in Figure 4. Fractions 1–7 were eluted by the pH 3.1 buffer and fractions 8–10 by the 0.5 M CH_3COONa solution. Electrophoresis showed that the first eluted fraction was composed of C secalins, eluted in the second fraction in chromatography of crude secalin on S200. The main constituents of fractions 2–8 were B secalins. A short time migration electrophoresis (not shown) demonstrated that fraction 9 was composed of the fast-moving constituents eluted in fraction 4 in gel filtration of crude secalin and fraction 10 was composed of peptides negatively charged at pH 3.2. As the slow-moving constituents (A secalins) eluted in fraction 4 in gel filtration of crude secalin on S200 (Figure 2) were also visible in some of the fractions 2–8 (diagrams of fractions 4– and 6, Figure 4), all these fractions were rechromatographed on Sephadex G-100 to separate A secalins from B secalins. All elution diagrams were similar to that of the fractionation of 4 SPS (Figure 5). B and A secalins were eluted in fractions 2 and 3, respectively. Small quantities of proteins which give trailings in starch gel electrophoresis (presumably low molecular weight glutelins) were also removed from SPS fractions by this procedure. A and B secalin fractions were ascribed the same number as the one of the SPS fraction from which they had been isolated: A_3 and B_3 secalins were isolated from fraction 3 SPS, A_4 and B_4 from fraction 4 SPS, and so on.

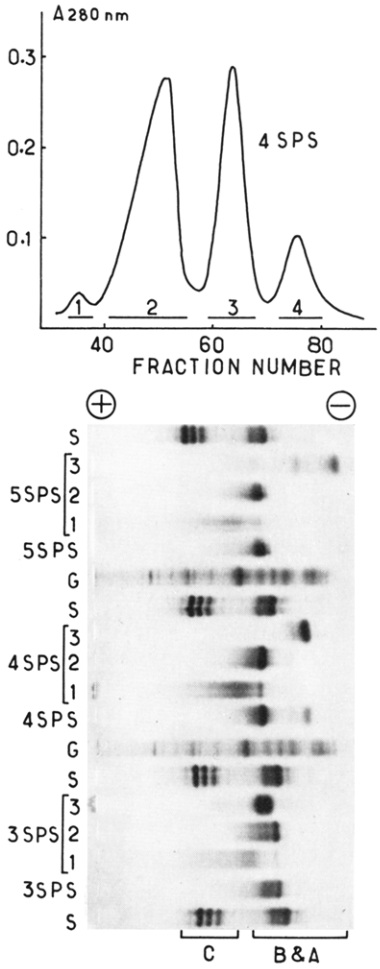

Figure 5. (Bottom) Starch gel electrophoresis at pH 3.2 of fractions isolated by rechromatography of SPS fractions on Sephadex G-100. (Top) Rechromatography of 4 SPS. S = crude secalin. G = crude Cappelle gliadin. 3, 4, and 5 SPS = SPS fractions (see Figure 4). 1, 2, and 3 = G-100 fractions.

A_4 and A_5 secalins migrated behind and in front of Cappelle α-gliadins, respectively, whereas A_3 secalins had the same velocity as slow β-gliadins.

Rye prolamins, like hordeins, the barley prolamins, are therefore distributed into three groups of constituents on the basis of their molecular size. This explains why we adopted the same nomenclature for secalins as the one proposed for hordeins (Køie et al., 1976; Shewry et al., 1977). As a matter of fact, hexaploid wheat gliadins are also distributed into three groups of constituents on the basis of their molecular weight since in these wheats, ω-gliadins have much higher molecular weights than γ, β-, and α-gliadins (Booth and Ewart, 1969; Charbonnier, 1974; Huebner et al., 1967; Kasarda et al., 1976; Tercé-Laforgue et al., 1980) and low molecular weight gliadins have also been isolated from wheat (Salcedo et al., 1979).

Characterization of Secalins. *Molecular Weights.* Fractions 2 and 3 isolated by fractionation of crude secalin on S200 were used for the determination of the molecular weight of secalins C and B, respectively, by gradient gel electrophoresis (Figure 6). One faint band with a M_r near 54 000 and one clear band of M_r 38 000 were visible in the diagram of fraction 2. As fraction 1 (S200) gave only a strong 54 000 band, the 54 000 band visible in the diagram of fraction 2 might have resulted from a contamination of fraction 2 by some fraction 1 constituents. Therefore, C secalin molecular weight was clearly lower than hexaploid wheat ω-gliadin and C hordein molecular weight (Booth

Table II. Amino Acid Composition of Secalin Fractions

	residues/1000 residues of secalin fractions											
amino acids	C	B_2	B_3	B_4	B_5	B_6	B_7	B_8	9 SPS	A_3[a]	A_4	A_5
Trp	0	3	2	4	4	4	3	4	15		5	6
Lys	4	7	7	7	7	7	7	8	22	2	0	2
His	5	11	10	13	14	14	16	17	17	1	0	1
Arg	18	14	18	14	18	18	16	16	46	25	33	32
Asx	4	20	26	27	27	27	27	27	55	27	22	29
Thr	10	24	31	26	33	30	26	27	39	77	79	73
Ser	44	52	53	55	57	54	51	52	51	74	74	75
Glx	436	378	355	353	345	346	344	340	188	274	257	264
Pro	288	208	177	188	179	185	191	189	120	87	73	81
Gly	12	23	23	22	23	23	24	24	75	60	60	58
Ala	5	24	27	26	26	27	27	27	52	58	64	63
½-Cys	1	24	28	27	28	27	27	28	62	83	86	78
Val	12	39	47	45	44	45	44	44	59	52	57	53
Met	0	11	16	16	15	15	16	15	20	44	46	37
Ile	41	45	55	55	53	52	56	55	44	45	51	53
Leu	38	62	71	71	72	70	71	72	78	45	47	49
Tyr	13	6	7	5	5	7	6	7	24	26	27	25
Phe	69	49	47	46	49	49	48	48	33	20	19	21

[a] Composition was determined on the basis of sulfur amino acid determination and 24-h hydrolysis results only.

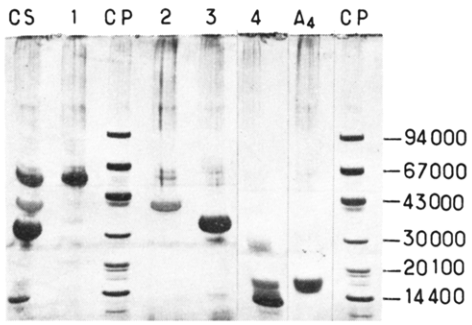

Figure 6. Secalin molecular weight determination by polyacrylamide gradient gel electrophoresis. CS = 60% EtOH crude secalin extracted from defatted flour. CP = calibration proteins (see Material and Methods). 1–4 = S200 fractions (see Figure 2). A_4 = A_4 secalin.

and Ewart, 1969; Charbonnier, 1974; Shewry et al., 1977; Brandt, 1976) though they could be considered as analogous to these proteins on the basis of their amino acid composition (see below).

Fraction 3 gave only a clear band with a M_r near 29 000 which is somewhat lower than the M_r or α-, β-, and γ-gliadins (Kasarda et al., 1976; Tercé-Laforgue et al., 1980; Huebner et al., 1967) and of some B hordeins (Shewry et al., 1977). The M_r of A secalins was found to be near 16 000. A secalin molecular weight is therefore in the same range as the molecular weight found for low molecular weight gliadins (Salcedo et al., 1979) and A hordeins (Mesrob et al., 1968; Mesrob and Petrova, 1969; Brandt, 1976; Shewry et al., 1977; Salcedo et al., 1980).

Amino Acid Analysis. The amino acid compositions of C, B, and A secalin fractions are given in Table II. Like ω-gliadins (Booth and Ewart, 1969; Charbonnier, 1974) and C hordeins (Shewry et al., 1980b), C secalins had high contents of Glx, Pro, and Phe and were sulfur amino acid free. The amino acid composition of all B secalins were very similar. However, because of slightly higher Glx and Pro contents, and lower Met contents, the B_2 fraction was somewhat different from other B fractions. As α-, β-, and, to a lesser degree, γ-gliadins are the only toxic gliadins for gluten-sensitive people (Jos et al., 1978), so are probably B secalins, which have amino acid compositions and molecular weights closer to α-, β-, and γ-gliadins than C secalins. Because of differences in amino acid composition between noxious gliadins and B secalins, the comparison of peptic–tryptic digests of these prolamins might give useful information about the identity of noxious peptides. As a matter of fact, these peptides certainly have the same structure whether isolated from wheat or from rye.

The amino acid composition of fraction 9 SPS was different from those of B fractions. Like starch gel electrophoresis, this composition showed that constituents of fraction 9 SPS could not be considered as secalins.

The adjustment of amino acid composition of A secalin fractions to the molecular weight determined by polyacrylamide gradient gel electrophoresis showed that arginine was the only basic amino acid present in these fractions. Moreover, A secalins had lower Glx, Pro, and Phe contents but much higher sulfur amino acid and Tyr contents than other secalins. Thus, differences between amino acid composition of A secalins and composition of other secalins are very similar to differences between low molecular weight gliadins and other gliadins (Salcedo et al., 1979).

N-Terminal Amino Acids. Arg, Asx, and Leu were found to be the N-terminal amino acids of C, B, and A secalins, respectively. There are therefore at least two main N-terminal sequences in rye prolamins since C and B fractions account for the 33.5 and 42.7% of the crude secalin precipitate, respectively (Table I). The N-terminal sequence found in Frontier rye prolamins was one of them (Autran et al., 1979). The other N-terminal sequence might be homologous with that found in a C hordein isolated from Julia barley (Shewry et al., 1980a) since both sequences have the same N-terminal amino acid.

CONCLUSION

The fractionation on Sephacryl S200 of precipitates of crude secalin extracted with 55% (w/w) *i*-PrOH or 60% (w/w) EtOH gives four fractions.

We think that proteins eluted in the first fraction should be considered as alcohol-soluble glutelins on the basis of their electrophoretic behavior in starch gel at pH 3.2.

Proteins eluted in fractions 2, 3, and 4, which migrated in the same region as wheat gliadins in starch gel electrophoresis at pH 3.2, were named C, B, and A secalins respectively.

Amino acid compositions of C secalins (M_r 38 000; N terminal is Arg) are very similar to ω-gliadin and C hordein amino acid compositions: high Glx, Pro, and Phe contents and lack of sulfur amino acids.

B secalins (M_r 29 000; N terminal is Asx) are characterized by the extreme similarity of their amino acid

composition, what suggests that they are homologous to each other, as found for wheat gliadins (Bietz et al., 1977).

As well as the amino acid composition of low molecular weight gliadins and A hordeins, the amino acid composition of A secalins (M_r 16 000; N terminal is Leu) is characterized by high Tyr and sulfur amino acid contents and a lack of Lys and His.

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Polyphenolic Changes in Ripening Bird-Resistant Sorghums

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Three chemical assays (vanillin–H_2SO_4, Folin–Denis, and cyanidin coloration), three biochemical assays (protein precipitation, α-amylase inhibition, and hemanalysis), and a paired preference assay on *Quelea quelea* were used to evaluate eight bird-resistant sorghum varieties in the milk, light dough, firm dough, and mature stages of grain development. Each assay showed an increase in the respective polyphenolic activity that peaked in the dough stages (usually firm dough) and then dropped sharply in the mature stage. Polyphenol activity in varieties classified as group II tended to peak earlier in grain development and then drop by a greater extent in the ripened grain. Although there is evidence that tannin biosynthesis goes to a higher degree of polymerization in group II than group III sorghums, the synthetic mechanism alone does not fully explain the differences between the two groups. Gel permeation, thin-layer, and paper chromatography analyses indicated that the tannins were procyanidins in both groups. Therefore, further elucidation depended upon differences in grain structure or the influence of other grain components. Three of these factors are discussed.

Sorghum is grown on 44 million hectares throughout the world (FAO, 1978), many of which are susceptible to extensive bird damage. Nearly all the sorghum grown in this country is used for livestock feed, but it is a staple food crop and the basic source of nourishment for the human population in many developing countries. Because of climatic conditions, farming practices, or marketing conditions, many farmers can grow only sorghums. Often, bird depredation further restricts the farmer's choices to brown, tannin-containing, bird-resistant (BR) varieties. Therein lies the dilemma; most brown sorghums available to farmers have unfavorable flavor and nutritional qualities. The U.S. standards for sorghums recognize them according to four classes: yellow, white, brown, and mixed ("Official United States Standards for Grain", 1974). Most of the

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