acid-precipitated soybean proteins (Koshiyama and Iguchi, 1965). Glucose, galactose, and mannose were the major sugars found in the isolates. Mannose was expected since it occurs in the 7S globulin glycoprotein (Koshiyama, 1969).

CONCLUSIONS

Sugars in soybeans exist in a diversity of forms including monosaccharides, oligosaccharides, polysaccharides, saponins, sterol glucosides, glycolipids, and isoflavones. By hydrolyzing the complex mixture and analyzing the resulting neutral sugars by the alditol acetate GLC procedure, we have obtained a greater insight than previously available about the individual sugars that comprise the carbohydrate fractions of soybean protein products. Our results confirm discrepancies noted earlier between the neutral sugar content obtained on hydrolysis of defatted soybean meal and the nitrogen-free extract (Kawamura, 1953). However, separate analyses for uronic acids and the oligosaccharides (to correct for fructose destruction) should give a fairly complete analysis of the total sugars and account for most of the nitrogen-free extract.

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Relationship among Maize Endosperm Characteristics of Normal and *Sugary Opaque-2* Kernels during Development

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A normal endosperm and its double mutant *sugary opaque-2* counterpart of a maize synthetic were compared for physical and chemical characteristics during 7 weeks at weekly intervals beginning 7 days after pollination. Nitrogen, potassium, magnesium, and phosphorus in the endosperm were determined and correlated with fresh weight, dry weight, and water content. Data indicated that nitrogen concentration is similar in both endosperms with the exception at 7 weeks after pollination (WAP). However, N content per endosperm was different in both endosperms from 3 to 7 WAP. Normal endosperms were consistently higher than *sugary opaque-2* endosperms in N content with maximum differences reaching 57% at late maturity. With a different pattern, potassium, magnesium, and phosphorus were significantly higher in concentration and content per endosperm in *sugary opaque-2* from 3 to 6 WAP. Nitrogen is highly correlated with endosperm dry weight for both normal and *su o2* endosperms. Potassium, Mg, and P showed high correlation with water content in both *su o2* and normal endosperms.

Genes affecting maize endosperm have been studied extensively in recent years. Special attention, however, has been given to *opaque-2* mutation since it increases in lysine content in the mutant endosperm (Mertz et al., 1964; Misra et al., 1972). Higher concentrations of K, P, Mg, Fe, and Zn have been found in *opaque-2* kernels than in normal seeds (Arnold et al., 1977a,b; Goodsell, 1968). *Opaque-2* endosperm shows also higher water content than normal endosperm from early stages of kernel development to maturity (Elmore, 1971). The *o2* effect on mineral content of the endosperm is still not well understood.

It was also shown elsewhere (Silva et al., 1978) that the *su* gene when combined with the *o2* gene enlarges the differences between normal and *opaque-2* endosperms for water-soluble polysaccharide and water content resulting

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Table I. Comparison between Normal and Double Mutant (*su o2*) Types for Nitrogen, Potassium, Magnesium, and Phosphorus Concentration[a] in Endosperm during Kernel Development

character	WAP[b]	endosperm type				significance level of the *t* value	difference as percentage of the normal mean
		normal	SD	*su o2*	SD[f]		
nitrogen, %	1	3.89	0.38	3.98	0.13	NS[c]	−2.3
	2	3.33	0.54	3.53	0.35	NS	−6.0
	3	2.03	0.22	1.83	0.24	NS	9.8
	4	2.11	0.37	1.87	0.32	NS	11.4
	5	1.89	0.37	1.88	0.33	NS	0.5
	6	1.82	0.32	1.70	0.22	NS	6.6
	7	1.72	0.34	1.66	0.24	*[d]	3.5
potassium, %	1	1.15		1.17			−1.7
	2	0.88	0.04	0.93	0.04	NS	−5.7
	3	0.81	0.02	0.94	0.04	**[e]	−16.0
	4	0.30	0.01	0.92	0.05	**	−206.7
	5	0.26	0.02	0.57	0.03	**	−119.2
	6	0.18	0.01	0.43	0.05	**	−138.9
	7	0.16	0.02	0.25	0.04	**	−56.2
magnesium, ‰	1	1.97		2.02			−2.5
	2	1.21	0.16	1.23	0.10	NS	−1.6
	3	0.51	0.06	0.69	0.04	**	−35.3
	4	0.24	0.04	0.48	0.07	**	−100.0
	5	0.21	0.04	0.39	0.06	**	−85.7
	6	0.18	0.04	0.30	0.08	*	−66.7
	7	0.13	0.03	0.24	0.04	**	−84.6
phosphorus, ‰	1	5.38		6.37			−18.4
	2	3.84	0.44	4.00	0.39	NS	−4.2
	3	1.75	0.21	2.44	0.18	**	−39.4
	4	0.92	0.16	1.82	0.20	**	−97.8
	5	0.78	0.25	1.58	0.29	**	−102.6
	6	0.50	0.13	0.78	0.23	*	−56.0
	7	0.36	0.15	0.60	0.07	*	−66.7

[a] On dry weight basis. [b] Weeks after pollination. [c] Nonsignificant at 5% level of probability. [d] Significant at 5% level of probability. [e] Significant at 1% level of probability. [f] Standard deviation.

in a high moisture and low dry weight per endosperm. By contrasting normal and *sugary opaque-2* endosperms on the same ear we thought would make a more precise study of possible relationships among physical and chemical characteristics of developing endosperms.

MATERIALS AND METHODS

The double mutant *sugary opaque-2* (*su o2*) corn variety developed by Silva et al. (1978) was used as the female parent in this study. The *su o2* plants were grown in adjacent 100-cm rows with a density of 50000 plants/ha in the experimental field of the Universidade Estadual de Campinas, São Paulo, Brasil. The plots were fertilized with N, P_2O_5, and K_2O at 60, 80, and 30 kg/ha, respectively. Through a split pollination technique the double mutant plants were selfed and outcrossed with a normal endosperm variety which was used in the synthesis of the double mutant variety (Silva et al., 1978). Normal (*Su su su o2 o2 o2*) and *sugary opaque-2* endosperms (*su su su o2 o2 o2*) were produced on the same ear in a proportion of 1:1 in approximately 105 adjacent plants.

Kernels from the middle portion of the ear were sampled for each genotype in groups of seven different plants, considering each plant a replication. The samples were taken at weekly intervals up to 7 weeks after pollination (WAP). Physiological maturity was attained at 6–7 WAP with black layer formation. A portion of kernels were degermed and used for fresh and dry weight determinations. For dry weight determination the endosperms were placed in an oven at 65 °C for 2 days.

For chemical determinations, 40 kernels of each genotype at each stage of development were degermed and stored frozen at −20 °C. The endosperms were then lyophilized and ground to powder using a Spexmil. The determinations of N and P were carried out in an autoanalyzer Technicon using a system according to Gerhke et al. (1973) for N and the molybdic phosphovandate method (Lott et al., 1956) for P. Potassium and Mg were determined by atomic absorption spectrophotometry.

RESULTS AND DISCUSSION

Means and standard errors for the normal and double mutant endosperm characteristics are given in Table I. The precision of the comparisons between the two endosperms may be considered as very high since the kernel types occur on the same ear and the environmental variance should have been minimal. Mineral concentration in the endosperm as a percentage of dry weight decreases throughout seed development in both endosperms. While N concentrations are practically identical in both endosperms, K, Mg, and P are significantly higher in the double mutant, except for the first 2 weeks after pollination. The differences may be as high as 200% for K, 100% for Mg, and 100% for P during intermediate stages of endosperm development. These results indicated that N has a different accumulation pattern from K, Mg, and P in the endosperm. Our observations are in agreement with those of Goodsell (1968) and Arnold et al. (1977a) that high lysine mutations might be responsible for the high percentage of K, Mg, and P in the endosperm.

New insights, however, may be given when one considers mineral content per endosperm instead of concentration. It can be seen from Figure 1A that fresh weight per endosperm is quite distinct in the two contrasting endosperms. While in the normal endosperm, fresh weight increased up to 7 WAP, the *su o2* endosperm reached a maximum around 5 WAP and then decreased rapidly at 6 WAP. Endosperm fresh weights in the *su o2* endosperm were significantly higher than in the normal endosperm, from 2 to 5 WAP, and significantly lower in the last 2 weeks of endosperm development.

Dry weight increased steadily during kernel filling, with

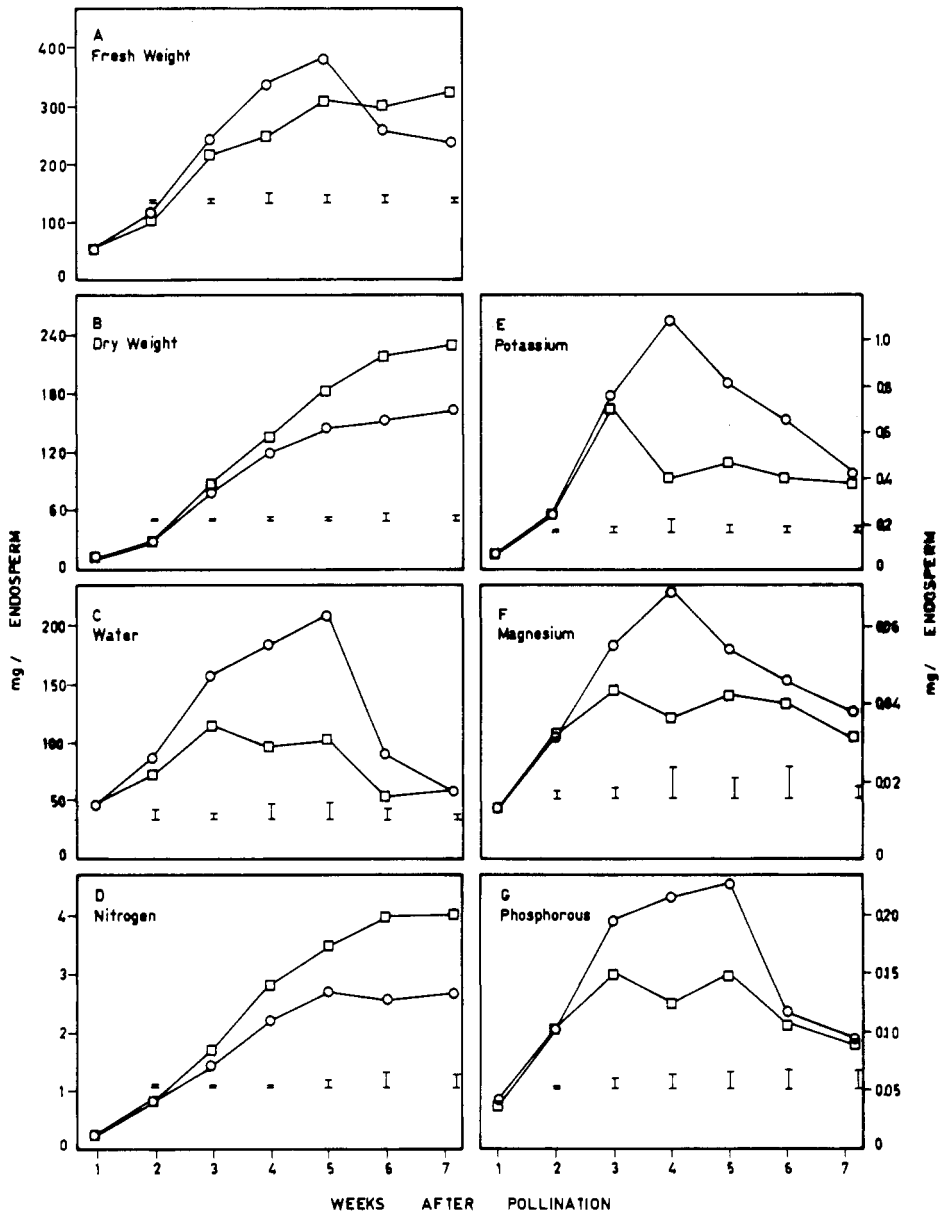

Figure 1. Comparison between normal (squares) and *sugary opaque-2* (circles) endosperms for amount of fresh weight, dry weight, water, nitrogen, potassium, magnesium, and phosphorus per endosperm, during endosperm development. Bars indicate LSD at 5% level of probability.

the normal endosperm showing a significantly higher dry matter accumulation rate than the double mutant (Figure 1B). While fresh weight in the double mutant can be as high as 30% of the normal endosperm (4 WAP), the later can accumulate dry weight 45% more than the double mutant endosperm at late maturity.

The water content per endosperm during maturity is shown in Figure 1C. Water in the *su o2* endosperm was 41, 92, 105, and 61% higher than in the normal type at 3, 4, 5, and 6 WAP, respectively.

While the content of N (Figure 1D) in the normal endosperm may be as much as 57% higher than in the *su o2* mutant, potassium (Figure 1E), magnesium (Figure 1F), and phosphorus (Figure 1G) in the latter may be respectively 170, 92, and 74% higher than the normal endosperm at 4 WAP. Thus N content per endosperm is maximum at late stages, while K, Mg, and P reach their highest values at intermediate stages of endosperm growth.

The relationships among physical and chemical characteristics of the endosperm may be easily seen by comparing the simple correlation coefficients for normal and double mutant shown in Table II. Nitrogen is highly correlated with endosperm dry weight, with $r = 0.99$ and $r = 0.98$, for normal and *su o2* endosperms, respectively, while K, Mg, and P are highly correlated with water

Table II. Correlation Coefficients among Physical and Chemical Characteristics of Normal (Upper Values) and of *Sugary Opaque-2* (Lower Values) Endosperms

	fresh wt	dry wt	water	N	K	Mg
dry wt	0.96					
	0.80					
water	0.32	0.05				
	0.83	0.34				
N	0.97	0.99	0.11			
	0.86	0.98	0.44			
K	0.63	0.43	0.82	0.45		
	0.90	0.61	0.85	0.66		
Mg	0.71	0.54	0.72	0.59	0.92	
	0.89	0.61	0.84	0.67	0.88	
P	0.58	0.36	0.89	0.42	0.89	0.95
	0.89	0.45	0.98	0.54	0.92	0.92

content in *su o2* and also normal endosperm.

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Major Proteins of Soybean Seeds. Reversible and Irreversible Dissociation of β-Conglycinin

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Reversible and irreversible dissociations of β-conglycinin were investigated by ultracentrifugation, disc electrophoresis, and immunodiffusion methods. The protein had a protomer conformation (7S) at high ionic strength ($I > 0.5$) or at acidic pH (pH <4.8) and a dimer conformation (10S) at low ionic strength ($I < 0.2$) in the pH region 4.8–11.0. Rapid interconversion between the protomer (trimeric structure) and the dimer (hexameric structure) was observed in the 0.2–0.5 ionic strength region. At very low ionic strength ($I < 0.01$), the α subunit dissociated from the protein. The dissociation was reversible but may result in the generation of multiple molecular forms (B_2 to B_6 conglycinins). The quaternary structures were stable at high ionic strength. Complete reversible dissociation into subunits occurred in 5 M urea ($I = 0.01$). Reversible dissociation into monomers (3–4S) appeared at pH 12.0 ($I = 0.5$). Dissociation into polypeptides (2S) at pH 2.0 and 12.0 ($I = 0.01$) was also reversible. Irreversible dissociation at pH 13.0 may be attributable to alkaline degradation.

Oligomeric storage proteins of legume seeds show a rather complicated reaction of association–dissociation. The ability to undergo conformational changes may have a physiological significance which relates to changes in osmotic pressure (Kretovich and Smirnova, 1960). The dissociation of the storage proteins has also been suggested to occur during seed germination to make the proteins accessible to proteinase attacks before final utilization by the seedling (Catsimpoolas et al., 1968). The dissociation of a protein into its protomers is, in most cases, reversible. Further dissociation of protomer into monomers is either reversible or irreversible depending on the properties of the protein and the condition under which the dissociation and, possibly, simultaneous unfolding of the monomers take place.

On the basis of association–dissociation properties, vicilin proteins (7S globulins) from legume seeds can be divided into three types (Derbyshire et al., 1976). One type dimerizes to a 9–12S form at 0.1 ionic strength and neutral pH, the second retains a 7S form at low ionic strength, and the third is insensitive to changes of ionic strength but associates to an 18S form (probably, a tetramer of 7S) at pH values near its isoelectric point. β-Conglycinin, a major 7S soybean globulin, belongs to the first type. γ-Conglycinin from soybeans has a characteristic of the second type (Koshiyama and Fukushima, 1976). A representative of the third type is G1 protein from *Phaseolus vulgaris* seeds (Sun et al., 1974).

Conformational changes of β-conglycinin have been reported (Naismith, 1955; Roberts and Briggs, 1965; Koshiyama, 1968). It is well-known that the conversion between protomer (7S) and dimer (10S) is reversible. However, dissociation of the protomer into subunits (polypeptide chains) has been unclear. Roberts and Briggs (1965) suggested that the protomer consists of at least seven different subunits. Koshiyama (1971) proposed nine subunits in a 7S molecule. We have isolated and characterized the constituent polypeptide chains (Thanh and Shibasaki, 1977) and presented trimeric structures for six different 7S molecules of β-conglycinin (Thanh and Shibasaki, 1978a,b).

From the new view on the subunit structure we investigated the association and dissociation of β-conglycinin at various pHs, ionic strengths, and urea concentrations using ultracentrifuge, disc electrophoresis, and immunodiffusion. The present study reveals a reversible dissociation into subunits at very low ionic strength and at acidic or alkaline pH and irreversible dissociation at extreme alkaline pH values. The findings are discussed with regard to the molecular structures of β-conglycinin.

MATERIALS AND METHODS

Protein Samples. β-Conglycinin, B_1 and B_6 conglycinins, and α, α′, and β subunits were isolated and purified as described previously (Thanh and Shibasaki, 1976a,b, 1977). The proteins were freeze-dried at pH 7.0.

Buffers. Potassium phosphate buffer at 0.1 ionic strength ($I = 0.1$) contained 2.6 mM KH_2PO_4, 32.5 mM K_2HPO_4, and 3 mM NaN_3, pH 7.8. Buffers at various ionic strength were prepared by adding NaCl ($I > 0.1$) or dis-

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