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CHAPTER 3

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Chemical Composition of the Seed

INTRODUCTION

Soybeans are well known for variations in color, size, and shape of the seed and other physical properties as well as their chemical composition. The physical and chemical differences are considerably modified by the heredity of the variety and the influences of the climatic conditions in which they are grown. Their photoperiodic characteristics or length of the day during the growing season is a major factor in controlling the onset of bloom and thus the yield of the plant. Accordingly, each variety produces best within a definite range of latitude.

Piper and Morse (1923) reviewed the early literature on soybeans and reported on the variations in oil and protein and on the iodine number of the oil as well as other factors resulting from varietal and environmental influences. Their report on 500 samples shows a range in protein content of 30–46% and in oil, of 12–24%. Another series of experiments by the USDA, reported by Dies (1942), on the characteristics of 128 varieties of soybeans shows the number of seeds per pound varied from 1232 to 9950, the oil content, from 13.9 to 23.2%, and the protein content from 32.4 to 50.2%. These data were from soybeans introduced from China, Manchuria, Korea, Japan, Siberia, France, and Italy during the years of 1880 to 1940 and grown in different locations in the United States.

During the long period of development of soybeans in China, Korea, Japan, and other oriental countries, the lack of travel and communication between farmers limited the exchange of information on methods of production and exchange of seed. This isolation led to the development of a great number of varieties and strains of soybeans. Piper and Morse (1923), who were responsible for a great many of the introductions from the Orient, stated "among the many varieties introduced from China and Manchuria, it is a very interesting fact that the same variety has rarely been secured a second time unless from the same place. It appears that practically every locality in these countries has its own local variety." To obtain the best varieties for the American farmer, more than 10,000 introductions were made for studies by the USDA and the state experiment stations in plant selection and breeding research.

In preliminary studies on the influence of fertilizer on the composition of soybeans, Cartter (1940) found that, while fertilizer level affected the yield of soybeans and varieties responded differently to different levels of application,

the fertilizer application had no noticeable effect on composition. He concluded that the importance of this work seems to be that composition is an inherited characteristic and thus makes possible breeding research on composition and yield as well as other agronomic characteristics.

NITROGENOUS CONSTITUENTS

Nitrogen Conversion Factor

In the analytical determination of protein in soybeans, soybean meal, and other protein products, a nitrogen to protein conversion factor is used. A true conversion factor for soybeans has not been determined. However, U.S. agronomists and commercial handlers and processors have used the value 6.25 since soybeans were introduced into the United States.

Jones (1931) published a list of proposed conversion factors for converting percentage of nitrogen in foods, feeds, seeds, and derived protein concentrates into percentage of protein in which he recommended a conversion factor for soybeans and soybean protein of 5.71. This factor was taken from the work of Osborne and Campbell (1898) in which they isolated and purified several protein fractions from the soybean. The fraction from which the above factor was taken contained 17.5% nitrogen and was called glycinin; it represented less than 10% of the total protein of the bean. Also, they isolated two smaller fractions, which they called legumelin and proteose, for which they reported nitrogen values of 16.12 each. The factor 5.71 originated from this early work and has been quoted frequently in the literature. However, the Osborne and Campbell (1898) report did not claim that their values represent the nitrogen content of the whole bean or any fractions other than those indicated; and, thus, their work does not justify the factor 5.71 for general use.

Agronomists and other analysts in the United States customarily use the factor 6.25 which is legally recognized by the National Soybean Processors Association, the Association of Official Analytical Chemists, the American Soybean Association, and other technical associations engaged in trade in soybeans and soybean products.

Research on soybean protein isolates by Smiley and Smith (1946) indicates that the factor 6.25 is probably higher than justified. Smith (1966) reviewed this problem and stated that "the use of two or more conversion factors in the literature will lead to confusion and difficulty in interpretation and comparison of nutritional and other data obtained in soybean protein investigations; and the use of a factor other than 6.25 will only introduce confusion and should be avoided."

Recently, Tkachuk and Irvine (1969) and Tkachuk (1969) made a different approach to the problem. They have determined nitrogen-conversion factors for a number of cereals and oilseed meals by using the amino acid composition of the meal protein as the basis for their calculations. They determined factors by dividing the weight of the amino acid residues, corrected for water, by the weight of the nitrogen they contain.

The use of the amino acid assay method of determining protein-conversion factors for protein isolates and concentrates is a decided improvement over the earlier methods, provided suitable corrections are made for nonprotein nitrogen (Becker *et al.* 1940) and other variables. To determine a protein factor for the whole seed, a further correction for the seed coat is necessary; the seed coat will vary with the size of the seed and its thickness and the correction will probably be a compromise. For the defatted soybean meal, containing the seed coat as well as the soluble nonprotein nitrogen, Tkachuk (1969) reported a conversion factor of 5.69. Perhaps one of the more important effects of the use of this smaller factor on soybean meal utilization will be in nutritional investigations which use the protein efficiency ratio (PER) for evaluating the protein. However, when the biological value (BV) or the ratio of the retained nitrogen to absorbed nitrogen is determined, a nitrogen to protein factor is irrelevant. In the nutritional evaluation (PER) of food preparations as well as animal feed formulations which are composites of several proteins, a factor for their particular combination of products would have to be determined.

Protein Composition of the Seed

One of the major investigations in the United States on the influence of variety, environment, and fertility level on the chemical composition of soybeans was reported by Cartter and Hopper (1942). A summary of their average results for the principal components of 10 varieties of soybeans grown at 5 locations for a 5-yr period is presented in Table 3.1.

Their data, on a moisture free basis, show average protein values for the 10 varieties range from 40.58% for the Peking to 46.42 for the Mandarin. Cartter and Hopper (1942) concluded from their investigation that the variation in protein content is the result of two factors: (1) locality where the beans are grown that is, soil and other environmental conditions; and (2) variety of the bean. Their report states that for well-composited soybeans the varietal factor is the greater and more consistent of the two. The soybeans in this investigation were not all commercial varieties and the average values for protein do not represent protein values for present-day commercial soybeans.

Notwithstanding the large number of soybean varieties from which U.S. growers may choose, only a few varieties are found in a given region. Their selection from the list of recommended varieties for the region are based largely on their adaptation to latitude, yield, and disease resistance. The recommended varieties for 1968 for the soybean growing areas from north to south are shown in Table 3.2. This table shows location numbers as well as protein and oil composition and seed weight of strains and varieties adapted to similar length of day and other location conditions. The varieties presently in production are

TABLE 3.1

AVERAGE COMPOSITION OF 10 VARIETIES OF SOYBEANS GROWN AT 5 LOCATIONS FOR A 5-YEAR PERIOD (TOTAL SUGARS AS SUCROSE)

Variety or Strain	Moisture Free Basis					
	Protein N X 6.25 (%)	Oil (%)	Total Sugar (%)	Crude Fiber (%)	Total Ash (%)	Iodine Number
Mandarin	46.42	18.16	6.76	5.39	5.37	127.6
Mukden	45.76	19.26	6.83	5.33	5.00	124.6
Dunfield A	41.38	20.97	8.24	5.34	4.65	124.9
Dunfield B	41.42	20.91	8.40	5.45	4.61	124.4
Illini	42.59	19.99	8.83	5.26	4.81	130.5
Manchu	44.06	19.40	7.78	5.42	5.12	130.2
Scioto	42.47	20.29	8.22	5.23	5.17	133.0
T-117	41.86	20.37	8.61	5.51	5.02	123.9
Peking	40.58	17.07	7.50	6.48	5.21	137.7
P.I. 54563–3	42.18	19.91	8.58	5.80	4.97	129.8

Source: Cartter and Hopper (1942).

subject to change as new varieties are developed in the agronomic program of breeding and selecting for improved composition, yield, and disease resistance.

High Protein Soybeans

When the soybean breeding program was initiated in the United States, the high price of oil in relation to the meal encouraged the plant breeders to develop high oil-yielding soybeans. As a result of this program, U.S. soybeans are higher in oil than beans from other countries, a characteristic which sometimes brings a premium over beans from other countries in the export markets. However, with increasing production of soybeans in the United States a large surplus of oil has developed which has caused a marked decrease in its price. It seems unlikely that in the United States the price of oil will ever regain its former position. Now, the dollar value of the meal is greater than that of the oil.

This change in price relationship has encouraged some of the plant breeders to revise their program to include the breeding of high protein soybeans. However, when the level of protein is raised other modifications in composition are brought into play such as a decrease in oil and carbohydrate and a loss in yield. Thus, the economics of the farmer and of the processor in choosing between high oil and high protein involves interrelated factors.

Krober and Cartter (1962) studied the interrelationship of the protein and the nonprotein constituents of the soybean such as oil, sugars, holocellulose, pentosans, crude fiber, ash and seed size. They found that the oil, sugars, and holocellulose were affected most by changes in protein content. When the protein was increased, they found that about 1/3 of the decrease in nonprotein constituents was in sugars, 1/3 in oil, and the remainder in holocellulose and

ANALYTICAL DATA FOR CURRENT SOYBEAN VARIETIES FROM UNIFORM TEST REPORT FOR 1968, NORTHERN GROUPS 00–IV AND SOUTHERN GROUPS IVS–VIII SUPPLIED BY USDA REGIONAL SOYBEAN LABORATORY URBANA, ILLINOIS
MOISTURE FREE BASIS

Uniform Test No.	Variety[1]	Hilum Color	Seed Weight	Protein N X 6.25 (%)	Oil (%)
00	Altona	Black	18.0	39.6	19.7
	Flambeau	Black	16.1	40.7	18.3
	Portage	Yellow	17.6	38.6	19.6
0	Clay	Yellow	17.0	40.2	21.1
	Grant	Black	16.7	40.0	19.7
	Merit	Buff	14.6	39.4	20.6
	Traverse	Yellow	18.0	40.7	19.8
I	Chippewa	Black	16.0	41.0	20.5
	Hark	Yellow	16.6	41.8	20.4
II	Amsoy	Yellow	17.2	38.7	22.0
	Corsoy	Yellow	15.9	39.6	21.5
	Harosoy 63	Yellow	18.0	40.3	21.1
III	Adelphia	Buff	15.8	39.2	21.7
	Calland	Black	17.8	38.7	21.3
	Wayne	Black	16.4	40.4	21.5
IV	Clark 63	Black	15.9	40.0	21.7
	Cutler	Black	18.0	40.4	21.6
	Kent	Black	17.7	40.0	22.0
IVS	Kent	Black	15.5	40.1	22.0
	Delmar	Yellow	14.3	39.2	22.2
	Custer	Black	13.7	36.9	22.0
V	Hill	Brown	11.8	38.5	21.2
	Dare	Buff	12.9	38.6	22.1
	York	Buff	17.3	38.6	20.9
	Dyer	Black	15.1	39.2	20.7
VI	Hood	Buff	13.4	39.3	21.7
	Lee	Black	12.4	40.6	21.1
	Lee 68	Black	12.5	40.5	20.9
	Pickett	Black	12.5	40.1	21.3
	Davis	Buff	13.4	38.8	21.6
VII	Bragg	Black	14.4	40.5	21.5
	Semmes	Black	14.3	41.4	20.9
VIII	Hampton	Buff	14.7	38.7	22.5
	Hardee	Buff	13.8	41.4	21.5

[1] Seed color of all varieties was yellow.

pentosans. However, the changes do not always follow the same pattern. In another lot of low protein beans, more than 1/2 of the increase was in oil with lesser changes in sugars and other constituents. The overall results indicate that the increase in protein was 2–3 times the decrease in oil and about 1/2 of the

loss in carbohydrates was in a form which is not readily digestible. They pointed out that in developing high protein beans it may be difficult to gain 3% protein with only 1% loss in oil while maintaining normal yields. These preliminary studies give indications there will be a compromise in the level of protein that can be bred profitably into soybeans.

Krober and Cartter (1966) devised experiments to separate, as far as possible, the genetic and environmental factors that affect protein content of the seed, especially the methionine, and to determine if in developing high protein beans there might be a change in the methionine which would affect nutritional value. They conducted four experiments with as wide a range of protein content and environmental conditions as can ordinarily be found and reported no significant tendency for methionine in the protein to decrease with increasing protein in the seed. They stated "in fact, there was more of a tendency toward a positive relationship, which is favorable to the development of high protein strains of good nutritional quality."

In calculating the value of the oil and meal derived from solvent extraction of 1 bu of soybeans, the yield of oil is approximately 10.7 lb and meal (44% protein) is 47.7 lb (Anon. 1971). The quotations on oil are usually in cents per pound and the meal in dollars per short ton. Using these data, the value of the oil and meal derived from 1 bu of beans can be estimated by multiplying the price of oil by 10.7 and the price of meal in dollars per ton by 0.0238 as the following example assuming the price of oil is 9c per lb and the meal $72.00 per ton:

10.7	× 0.09	= $0.963 (oil)
72.00	× 0.0238	= 1.713 (meal)
Total		$2.676 per bu

These data can be used along with the yield to estimate the value of the oil and meal derived from an acre of soybeans. With these and other cost data, an estimate can be made at what price of oil and meal an economic advantage can be gained by changing to production of high protein soybeans.

However, in estimating the future trends, the world price of oil must be given consideration. In many countries the price of oil still remains at a high level; thus the production of high oil soybeans for the export market will need to be considered. With a possible need for high oil beans for the export market and high protein beans for the domestic specialty products market, soybean production might be divided between the two types of soybeans.

Garden Type Soybeans

Garden types are soybeans which the Chinese and other oriental people use during the summer as green beans for the table. They were introduced into the U.S. program and tested as a potential garden crop by Lloyd and Burlison (1939), Woodruff and Klaas (1938), and Weiss *et al.* (1942). The garden type

soybeans are sometimes referred to as vegetable or edible soybeans; however, at present the most popular designation is "garden type." The garden varieties can be preserved by freezing and canning much like other vegetables.

Garden type soybeans are not basically different from field varieties but are reported generally to be larger in size, higher in protein, lower in oil, lower in yield, and on reaching maturity they have a tendency to shatter from the pod, resulting in substantial loss if harvested with a combine. Garden varieties are reported to have a better flavor and texture than the regular field beans and have been compared in these qualities to lima beans.

Table 3.3 gives the protein and oil content of several varieties of garden type beans and Table 3.4 compares garden type beans with other common beans and with peas. The garden type contains about twice as much protein as the other beans and peas and 11 times as much oil. Thus, they are much higher in nutritive and caloric value than other garden beans and peas.

Nonprotein Nitrogen

Soybeans contain small quantities of peptides and amino acids having variable molecular dimensions which may occur as the residue of incomplete protein synthesis or possibly the result of protein degradation. Muller and Armbrust

TABLE 3.3

PROTEIN AND FAT CONTENT AND SEED SIZE OF 12 VARIETIES OF MATURE GARDEN TYPE SOYBEANS TESTED AT URBANA, ILLINOIS (MOISTURE FREE BASIS)

Variety	Protein N × 6.25 (%)	Oil (%)	Weight Avg 3 Yr (Gm/100 Seed)
Very early			
Giant green	39.3	22.4	29.4
Early			
Bansei	36.4	21.6	21.2
Fuji	39.6	21.4	25.9
Midseason			
Illini	38.7	21.4	13.9
Hokkaido	40.4	20.8	31.9
Jogun	40.7	19.9	29.9
Willomi	42.3	19.4	31.1
Late			
Illington	42.9	18.6	25.9
Imperial	41.0	20.5	28.4
Funk Delicious	42.3	20.0	31.7
Emperor	42.2	19.9	29.7
Higan	41.0	18.1	23.4

Source: Lloyd and Burlison (1939).

TABLE 3.4

COMPOSITION OF GARDEN TYPE SOYBEANS AND OTHER VEGETABLE BEANS AND PEAS

Food	Moisture (%)	Protein N X 6.25 (%)	Fat (%)	CHO Total (%)	Ash (%)	Calcium (%)	Iron (%)	Calories (per/Lb)
Green shelled								
Soybeans	70.0	12.2	5.2	11.1	1.52	0.072	0.0029	636
Lima beans	66.5	7.5	0.8	23.5	1.71	0.028	0.0024	595
Broad beans	74.1	8.1	0.6	15.8	1.40			460
Peas	74.3	6.7	0.4	17.7	0.92	0.028	0.00207	460
Mature dry								
Soybeans	7.0	40.6	16.5	30.9	5.0	0.212	0.0103	1973
Lima beans	10.4	18.1	1.5	65.9	4.1	0.071	0.0086	1586
Navy Beans	12.6	22.5	1.8	59.6	3.5	0.158	0.0079	1564
Peas	9.5	24.6	1.0	62.0	2.9	0.084	0.0057	1612
Flour								
Soybean	5.1	42.5	19.9	24.3	4.5	...	...	2026
Wheat	12.4	11.2	1.0	74.9	0.5	0.021	0.0008	1603

Source: Woodruff and Klaas (1938).

(1940) reported that a protein-free extract of mature soybeans contained adenine, arginine, choline, glycine, betaine, trigonelline, guanidine, tryptophan, and probably canavanine. Glutathione, quaternary amines and other organic nitrogen-containing compounds have been qualitatively identified. These minor nitrogen bearing compounds are classified as nonprotein nitrogen (NPN) of the soybean.

The peptides have a wide variation in molecular dimension, and there is no sharp line which distinguishes a large peptide from a low molecular weight protein molecule: thus, the level of the nonprotein nitrogen constituents in soybeans is a somewhat arbitrary value and is influenced by the method used in its determination.

Becker *et al.* (1940) developed a simple method for the determination of NPN in soybeans which is based on the extraction of either the defatted or full-fat soybeans with 0.8 N trichloroacetic acid. The result from meal ground in a Wiley mill to pass a ½ mm screen was the same as for meal ground in a hammer mill to pass through a 100 mesh screen. They found also that results with 0.8 N trichloroacetic acid were essentially the same as those obtained by using a method which separated the NPN from the protein and other constituents by dialysis through a selected cellophane membrane. Their data show that the concentration of trichloroacetic acid is not critical since TCA values ranging from 0.65 N to 1.0 N give essentially the same results. The value of 0.8 N trichloroacetic acid was taken as the midpoint of this range.

Becker *et al.* (1940) determined the NPN in fat free meal from 12 varieties and strains of soybeans ranging in protein content from a high of 56.4% to a low of 41.4%. Also, they compared NPN values for germinated and ungerminated Dunfield beans. These results are shown in Table 3.5. Their data show a range of NPN from a high of 7.80% to a low of 2.88% based on the weight of the fat free meal. It is apparent from these data there is no correlation between the amount of total nitrogen in the meal and the NPN. The germinated seed contains double the amount of NPN as the ungerminated seed.

Krober and Gibbons (1962) investigated the relationship of the environmental factors which might be expected to influence the level of NPN as well as the relationship of NPN to the level of protein in the seed. In general, they found that the slight tendency of the NPN to increase with increasing protein was too small to be significant and most of their data showed very little effect of location or variety on NPN. The major influence on mature beans appeared to be weather conditions. Unfavorable weather conditions, whether too cold and wet or too hot and dry, were associated with a high percentage of NPN. These

TABLE 3.5

NONPROTEIN NITROGEN
EXTRACTION OF FAT FREE MEAL WITH 0.8 N TRICHLOROACETIC ACID

	Fat-free Meal			Whole Meal		
	Total N/gm of Meal (Mg)	0.8 N N/gm Meal (Mg)	Extract N Extracted (%)	N (%)	Oil (%)	Moisture (%)
Mukden 1936 (Iowa)	90.3	7.04	7.80	7.68	16.48	6.45
Mukden 1937 (Ind.)	84.9	3.10	3.65	7.07	17.30	5.75
Dunfield 1937 (Ohio)	73.2	2.11	2.88	6.00	19.50	5.48
Mukden 1937 (Ohio)	79.8	2.30	2.88	6.77	18.08	5.78
Mukden 1937 (Iowa)	83.1	3.92	4.72	7.00	18.39	5.83
Dunfield 1937 (Iowa)	76.0	2.78	3.66	6.13	19.92	5.74
Illini 1937 (Ark.)	66.2	3.07	4.64	5.03	23.79	5.53
86518 1937 (Iowa)	85.7	6.38	7.44	7.50	15.15	6.10
Dunfield 1937 (Iowa)	70.3	2.61	3.71	5.72	21.24	5.85
Mukden 1937 (Iowa)	78.0	2.74	3.51	6.54	18.60	5.77
Dunfield 1937 (Ind.)	79.3	5.02	6.33	6.89	14.47	6.00
Dunfield 1937 (Ind.)	73.0	2.50	3.42	6.07	20.64	5.67
Ungerminated Dunfield 1937	77.8	2.81	3.61			
Germinated Dunfield 1937	77.8	5.63	7.24			

Source: Becker *et al.* (1940).

)nditions may have influenced the proper maturing of the seed. Krober and ollins (1948) reported, also, that weather-damaged beans often are higher in PN than undamaged beans.

itrogen Distribution in Meal Fractions

The defatted soybean meal and its fractions are the basic raw material for reparing or supplementing many food products and mixed feeds and for the reparation of protein isolates, concentrates, and less important residues. Rackis *: al.* (1961) processed defatted meal (obtained from 1958 Hawkeye soybeans) n a laboratory scale into various fractions and determined their relative percentges as well as their nitrogen and protein content; their fractionation procedure outlined in Fig. 3.1, and the analytical data are given in Table 3.6. The data ccount for 96.3% of the nitrogen in the defatted meal; however, the nonprotein itrogen was lost during dialysis and recovery of the whey.

Because soybeans vary in composition, and also because the work was perormed on a laboratory scale, the results will be somewhat different from similar ata obtained in large-scale processing of the meal. Nevertheless, the results give n approximation of the nitrogen distribution when the seed is fractionated for roduct development. For example, when the process is carried out on a ommercial scale to produce acid precipitated protein, the yield will be 4–6% ower than the 36.9% obtained for the laboratory procedure; and most of the rotein which does not appear in the protein isolate will be found in the residue nd whey fractions. However, according to the results of Krober and Cartter

TABLE 3.6

YIELD, NITROGEN, AND PROTEIN CONTENT OF SOYBEAN MEAL FRACTIONS (DRY BASIS)

Fraction	Yield Gm/100 Gm Meal	Nitrogen (%)	Protein (%)	Percentage Total N
Soybean meal	100.0	9.83	61.4	–
Acid-precipitated protein	36.9	16.29	101.9	61.1
Residue	30.3	8.31	52.0	25.6
Total whey solids	31.9	2.86	17.9	9.3
Isolated whey protein	3.9	16.23	101.4	6.4
Phytate-protein complex	0.93	2.98	18.6	0.3
Seed coat	8.0	1.53	9.56	–
Hypocotyl, whole seed basis	2.0	7.90	49.40	–
Acid-precipitated protein of hypocotyl		15.19	95.15	–

Source: Rackis *et al.* (1961).
Total nitrogen recovered 96.3%

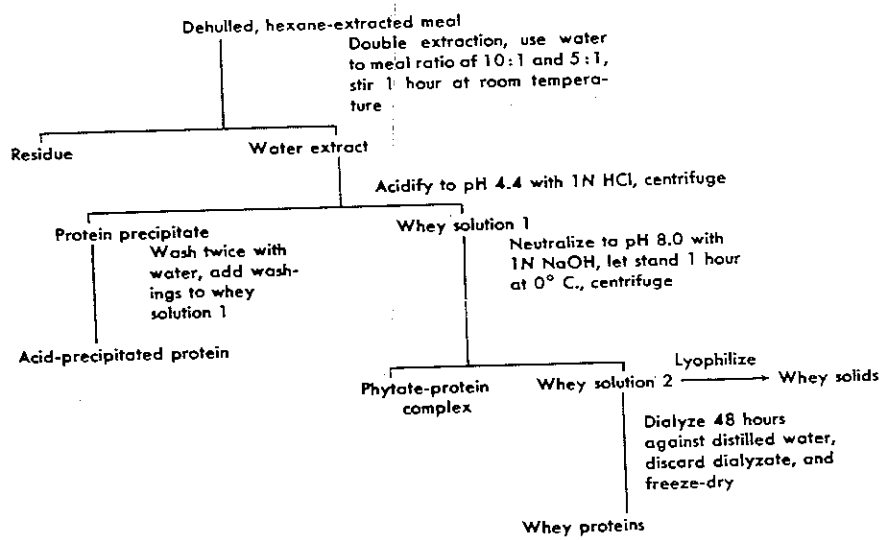

FIG. 3.1. PREPARATION OF SOYBEAN MEAL FRACTIONS

(1962), the variations of nitrogen in the fractions will be influenced more by the mechanics of fractionation than changes in the original level of protein in the soybean.

Amino Acid Distribution in Meal Fractions

In processing defatted meal for producing specialty products as illustrated in Fig. 3.1 there occurs a nonuniform distribution of amino acids in the various fractions which is given in Table 3.7. For example, the data show that the essential amino acids in the acid-precipitated protein are lower than in the meal. This is especially true for the lysine, tryptophan, threonine, methionine, and cystine; the values for the tryphophan, cystine, and cystine plus methionine are 20% or more lower in the protein isolate than in the meal. In the whey protein the histidine, lysine, tyrosine, threonine, cystine, and methionine values are 20% higher than in the meal. This amino acid fractionation is an important nutritional consideration in formulating the meal fractions into food products.

SOYBEAN OIL

It has been demonstrated that the oil content of soybeans and the composition of their fatty acids are influenced by the genetic characteristics of the variety and the climatic environment during the period in which the oil is elaborated. The extremes in fatty acid composition and iodine values of soybean oils are illustrated in Table 3.8 by data taken from Dollear *et al.* (1940).

TABLE 3.7
AMINO ACID COMPOSITION OF SOYBEAN MEAL FRACTIONS

Amino Acid	Whole Meal	Residue	Acid-precipitated Protein	Whey Protein	Hulls	Hypocotyl Meal	Acid-precipitated Protein of Hypocotyl
	(Grams of Amino Acid/16 Gm N)						
Arginine	8.42	7.44	9.00	6.64	4.38	8.32	6.38
Histidine	2.55	2.70	2.83	3.25	2.54	2.60	2.65
Lysine	6.86	6.14	5.72	8.66	7.13	7.45	7.80
Tyrosine	3.90	3.30	4.64	4.67	4.66	3.48	3.78
Tryptophan	1.28	—	1.01	1.28	—	—	—
Phenylalanine	5.01	5.24	5.94	4.46	3.21	3.88	4.22
Cystine	1.58	0.71	1.00	1.82	1.66	1.24	—
Methionine	1.56	1.63	1.33	1.92	0.82	1.72	1.79
Serine	5.57[1]	5.97[1]	5.77[1]	7.62[1]	7.02[1]	4.90[1]	4.50[1]
Threonine	4.31	4.67	3.76	6.18	3.66	4.00	3.82
Leucine	7.72	8.91	7.91	7.74	5.93	6.62	7.22
Isoleucine	5.10	6.02	5.03	5.06	3.80	4.11	4.53
Valine	5.38	6.37	5.18	6.19	4.55	4.82	5.28
Glutamic acid	21.00[1]	17.76[1]	23.40[1]	15.64[1]	8.66	13.78	14.12
Aspartic acid	12.01	12.39	12.87[1]	14.08[1]	10.05[1]	9.74	9.84
Glycine	4.52	5.21	4.56	5.74	11.05	4.25	4.93
Alanine	4.51	5.73	4.48	6.16	3.98	4.69	4.47
Proline	6.28	5.35	6.55	6.66	5.76[1]	4.23	4.38
Hydroxyproline	0	0			7.57[1]	Trace	0
Ammonia	2.05	2.61	2.20	1.53	1.55[1]	1.40	1.20

[1] Values obtained by extrapolation to zero-hydrolysis time.

TABLE 3.8
IODINE NUMBERS AND FATTY ACIDS OF SOYBEAN OIL

Variety and Location	Year	Iodine No.	Saturated Acids (%)	Unsaturated Fatty Acids			
				Total (%)	Oleic (%)	Lino-leic (%)	Linolenic (%)
Dunfield (Mo.)	1936	102.9	12.0	88.0	60.0	25.0	2.9
Dunfield (Mo.)	1937	124.0	13.2	86.8	34.0	49.1	3.6
Dunfield (Ind.)	1937	127.3	13.1	86.9	34.8	46.0	6.0
Illini (Ill.)	1936	131.6	12.7	87.3	27.7	53.7	5.9
Peking (Ill.)	1937	137.8	12.4	87.6	24.4	56.2	7.3
Seneca (N.Y.)	1938	139.4	11.9	88.1	27.4	55.4	8.0
Wild beans	1938	151.4	13.5	86.5	11.5	63.1	12.1

Source: Dollear *et al.* (1940).

They reported that the ratio of saturated to unsaturated fatty acids is fairly constant, irrespective of the total amount of oil present in the seed or of the iodine number of the extracted oil.

The average oil content of 10 varieties of soybeans grown at 5 locations during a 5-yr period in the midwest area were reported by Cartter and Hopper (1942) (Table 3.1). The average oil content for the 10 varieties on a moisture free basis is 19.63%. If the noncommercial varieties are eliminated the average is increased slightly to 19.98%. Their results show that the effect of location is as important as the effect of variety on oil content. The oil content of presently recommended soybean varieties is given in Table 3.2.

Table 3.8 shows the principal fatty acid and iodine number of the wild soybean, which is generally recognized as the precursor of present-day beans. Other compositional date of the wild soybean, taken from Dollear *et al.* (1940), are tabulated below:

	%		%
Nitrogen	7.92	Potassium	1.77
Protein N X 6.25	49.50	Phosphorus	0.91
Ash	6.88	Calcium	0.38
Lipids	5.4	Polysaccharides	
Crude fiber	10.35	as sucrose	5.62

The most notable differences between present-day soybeans and the wild bean are the high protein and fiber and very low oil of the wild bean.

Crawford and Gillingham (1967) made a 2-yr study on the effect of variety and location on the oil and protein content of soybeans in South Carolina. They found that location within the area was a major factor in affecting oil content and they also concluded that oil content may be determined predominantly by

variety. They recommended that highest yields will be obtained by planting a given variety in a selected location. However, their report states that location was without effect on protein content.

Howell and Collins (1957) made a study on the effect of environment on the variability of the linolenic and linoleic acid. They found that differences in temperature caused major differences in linolenic and linoleic acid content of soybean oil; they stated that differences associated with temperature are of greater magnitude than associated with variety.

Collins and Sedgwick (1959) studied the fatty acid composition of several varieties of soybeans. The soybean seed samples used in their study were taken from the Uniform Test Groups 0 through VIII of the U.S. Regional Soybean Laboratory program, and grown in 6 to 14 locations. Their results are summarized in Table 3.9.

In the 2 crop years of the study, the soybean oil for the whole series ranged from 5 to 11% in linolenic, 43 to 56% in linoleic, 15 to 33% in oleic, and 11 to 26% in saturated acids. In general, when soybean varieties were grown near the northern range of their area of adaptation, they produced oil which was 1–2 percentage points higher in linolenic and 3–6 percentage points higher in linoleic

TABLE 3.9

FATTY ACID COMPOSITION AND IODINE VALUES OF SOYBEAN OIL FOR YEARS 1956–1957 in FOUR UNIFORM TEST GROUPS

Uniform Test Group	Linolenic Acid (%)	Linoleic Acid (%)	Oleic Acid (%)	Saturated Acids (%)	Iodine Value (%)
I					
Avg 5 varieties	8.59–8.76	48.0–48.4	23.7–23.4	19.5–19.4	131.9–132.7
High-low	9.45–8.19	51.2–45.9	26.4–21.0	20.0–18.9	135.2–129.7
III					
Avg 6 varieties	7.87–8.14	49.3–49.3	23.6–22.8	19.2–19.7	132.0–131.9
High-low	8.70–7.25	51.4–46.0	26.7–21.1	20.7–18.8	134.2–128.9
IV					
Avg 5 varieties	8.14–8.06	49.1–47.8	23.9–26.5	18.9–17.7	132.8–132.2
High-low	8.72–7.46	51.2–45.0	29.3–23.1	19.7–15.1	137.0–136.3
VII					
Avg 3 varieties	7.24–8.02	51.2–54.0	22.9–19.4	18.6–18.6	133.1–136.9
High-low	8.49–6.73	54.9–49.5	26.6–18.2	19.8–17.3	138.0–131.9

Source: Collins and Sedgwick (1959).

acid than when grown at the southern range of their adaptation. Within each group, varieties tended to maintain the same relative order of fatty acid composition of oil at all locations in the 2-yr study.

ASH AND MINERAL CONSTITUENTS

The most extensive investigation of the ash content of soybeans, including the minerals, potassium, phosphorus, and calcium, is that reported by Cartter and Hopper (1942). They investigated 10 varieties, grown at 5 locations, for a 5-yr period; a summary of their average results for ash, potassium, phosphorus, and calcium is given in Table 3.10. The original data show that the ash of 1 of the varieties, Mandarin, was consistently high for the 5-yr period; its highest value for a single sample and for the series was 5.90% and its average for the series was 5.37%. Another variety, Dunfield, was consistently low and had a minimum of 3.67%, which was also low for the series; its average was 4.65%. The average for the 10 varieties for the total period was 4.99%.

The principal mineral components of the ash as reported by Beeson (1941) are shown in Table 3.11. The average values are potassium, 1.83%; phosphorus, 0.78%; magnesium, 0.31%; and sodium, calcium and sulfur, each 0.24%. Since the composition of the ash is made up of the residue of many components of the seed, and since the portion of each component may be influenced in a different degree by variety, climatic, and soil conditions, it is to be expected that the identification of the specific factors which control their concentration would be

TABLE 3.10

AVERAGE ASH, PHOSPHORUS, POTASSIUM, AND CALCIUM FOR 10 VARIETIES OF SOYBEANS GROWN AT 5 LOCATIONS FOR 5 YEARS

Variety or Strain	Moisture-free Basis			
	Ash (%)	Phosphorus (%)	Potassium (%)	Calcium (%)
Mandarin	5.37	0.696	1.64	0.386
Mukden	5.00	0.660	1.74	0.240
Dunfield A	4.65	0.626	1.62	0.226
Dunfield B	4.61	0.627	1.58	0.221
Illini	4.81	0.623	1.67	0.252
Manchu	5.12	0.670	1.67	0.313
Scioto	5.17	0.658	1.68	0.343
T-117	5.02	0.654	1.67	0.248
Peking	5.21	0.727	1.75	0.272
Boone	4.97	0.653	1.71	0.253
Average	4.99	0.659	1.67	0.275

Source: Cartter and Hopper (1942).

TABLE 3.11

MINERAL CONTENT OF SOYBEANS (MOISTURE FREE BASIS)

Mineral	No. of Analyses	Range		Mean (%)
		Maximum (%)	Minimum (%)	
Ash	...	6.35	3.30	4.60
Potassium	29	2.39	0.81	1.83
Calcium	9	0.30	0.19	0.24
Magnesium	7	0.34	0.24	0.31
Phosphorus	37	1.08	0.50	0.78
Sulfur	6	0.45	0.10	0.24
Chlorine	2	0.04	0.03	0.03
Sodium	6	0.61	0.14	0.24
Boron	5	0.0029	0.0006	0.0019
Manganese	11	0.0041	0.0021	0.0028
Iron	13	0.0133	0.0057	0.0080
Copper	1			0.0012
Barium	...			0.0008
Zinc	1			0.0018

Source: Beeson (1941).

difficult to identify and to substantiate. However, Cartter and Hopper (1942) did find consistent differences in ash content based on location, which they concluded were mostly the effect of soil and climate. They concluded, also, that the factors of environment which influenced oil and protein metabolism do not influence total ash accumulation in the seed in the same manner.

Smirnova and Lavrova (1934), reporting earlier on the composition of soybeans, stated that the composition of the ash varies and at the same time the level of the phosphorus varies in direct production to the total amount of ash.

Cartter and Hopper (1942) show a range of potassium for the 10 varieties extending from a high of 2.17% to a low of 1.29% with an average value of 1.67%. This is a narrower range than the values of 2.39 and 0.81% reported by Beeson (1941). When Cartter and Hopper compared their results for potassium with that for phosphorus, they found that seasonal variations had more effect on the metabolism of potassium than on phosphorus.

Cartter and Hopper, in their investigations on calcium in 10 varieties, found a low of 0.163%, a high of 0.470%, and a mean of 0.275%. They found a tendency for the calcium in soybeans to be above average during warm seasons and concluded that temperature plays an important part in determining the amount of calcium stored in the soybean.

PHOSPHORUS CONSTITUENTS

The compounds which contribute phosphorus to the soybean are inorganic phosophorus, phytin, several different phospholipids, and nucleic acids. Analytical investigations show a wide variation in the phosphorus content of soybeans as reported in the ash. Cartter and Hopper (1942) determined the phosphorus content of 10 varieties of soybeans grown over a 5-yr period. Their results (Table 3.10) range from an average low of 0.623% for the Illini variety to an average high of 0.727% for the Peking, with a mean value of 0.659%. In a single year the average low was 0.419% for the Mukden and the high 0.830% for the Peking. For this latter series, the high is approximately double that of the low value.

Phytin and Inorganic Phosphorus

The principal source of phosphorus in soybeans, as in most seeds, is phytin, the calcium-magnesium-potassium salt of inositol hexaphosphoric or phytic acid (Staley Mfg. Co. 1952). The phytates are especially important because of their effect on protein solubility and calcium nutrition. Averill and King (1926) found the phytin phosphorus content of soybeans to vary from 0.505 to 0.727%.

Pons and Guthrie (1946) determined the total and inorganic phosphorus in a number of plant materials, including defatted soybeans, cottonseed, and peanut meals. Their results are in Table 3.12. Also, they show phytin values for

TABLE 3.12

INORGANIC PHOSPHORUS CONTENT OF VARIOUS PLANT MATERIALS (DRY BASIS)

	Total Phosphorus (%)	Inorganic Phosphorus		
		1-hr Extraction (%)	24-hr Extraction at 25° C. (%)	24-hr Extraction at 5° C. (%)
Cottonseed meal				
diethyl ether extracted	1.722	0.085	0.085	0.085
Skellysolve B extracted	1.711	0.071	0.073	0.071
Peanut kernels				
Skellysolve F extracted	0.849	0.081	0.082	0.079
Raw cotton fiber	0.028	0.015	0.015	0.015
Sweet potatoes, L-5	0.135	0.075	0.076	0.076
Jerusalem artichokes	0.385	0.083	0.085	0.085
U.S. 13 corn	0.282	0.016	0.016	0.016
Milo	0.274	0.016	0.018	0.017
Federation wheat	0.377	0.018	0.019	0.019
Kharkov wheat	0.445	0.017	...	...
Wheat straw	0.165	0.118	0.122	0.119
Soybean meal				
Skellysolve F extracted	0.750	0.036	0.037	0.036
Phytin, crude				
from peanuts	14.31	0.070	...	...
from cottonseed	13.84	0.061	...	...
Dialyzed peanut protein	0.650	0.014	...	...
Dialyzed cottonseed protein	1.164	0.013	...	...

Source: Pons and Guthrie (1946).

cottonseed and peanut meals and total phosphorus for dialysed, isolated cottonseed and peanut proteins.

The cottonseed meal with 1.72% of total phosphorus has more than double the value of the soybean meal; however, the value of 0.849% for the peanut meal is nearly the same as for the soybean. The crude phytin values of 13.84% for cottonseed and 14.31% for peanut are more than double the values reported for soybeans by Averill and King (1926). For the dialysed, isolated cottonseed protein, Pons and Guthrie (1946) reported 1.164% phosphorus which is much higher than the 0.8–1.0% found in isolated soybean protein by Smith and Rackis (1957).

Earle and Milner (1938) investigated the effectiveness of various solvents for extracting the phosphorus-containing compounds from soybeans as shown in Tables 3.13 and 3.14. Of the total phosphorus in the Dunfield variety, which they studied, they found that phytin phosphorus accounts for approximately 75%; the phosphatide phosphorus, 12%; the inorganic phosphorus, 4.5%; and the residual phosphorus, 6%. It is possible that the residual phosphorus is nucleic acid phosphorus, which they did not report. When they extracted with petroleum ether, most or perhaps all of the phytin phosphorus remained in the defatted meal. They found that only about 0.5% of the total phosphorus, which was

TABLE 3.13

PHOSPHORUS REMOVED FROM SOYBEANS BY SUCCESSIVE EXTRACTION USING VARIOUS SOLVENTS

Solvent	Type Phosphorus Removed	Phosphorus[1] Removed from Samples Numbered 1 (Mg)	2 (Mg)	3 (Mg)	4 (Mg)	5 (Mg)	6 (Mg)
Petroleum ether	Phosphatide	—	—	0.02	0.02	0.03	0.03
Alcohol, 95%	Phosphatide	0.80	0.77	—	—	0.71	0.71
Filter paper from above		0.11	0.11	0.13	0.13	0.14	0.15
Alcohol-HC1, 1st extraction	Inorganic	0.20	0.19	0.76	0.75	0.17	0.19
Alcohol-HC1, 2nd and 3rd extractions	Inorganic	0.06	0.07	0.11	0.12	0.08	0.08
Filter paper from above		0.12	0.12	0.16	0.13	0.09	0.10
HC1, 1.8% in water, 1st extraction	Phytin	4.09	3.86	3.86	3.92	3.90	3.86
HC1, 1.8% in water, 2nd and 3rd extractions	Phytin	0.15	0.18	0.33	0.15	0.23	0.19
Phosphorus in residue		0.23	0.31	0.34	0.35	0.31	0.30

Source: Earle and Milner (1938).

[1] Results expressed in milligrams of phosphorus per gram of whole bean. Total phosphorus in beans 6.02 mg per gram.

TABLE 3.14

DISTRIBUTION OF PHOSPHORUS IN DUNFIELD SOYBEANS CONTAINING 6.02 MG PHOSPHORUS PER GRAM OF WHOLE BEAN

Sample No.	Phosphatide Phosphorus (Mg)	Inorganic Phosphorus (Mg)	Phytin Phosphorus (Mg)	Phosphorus in Residue (Mg)	Total Accounted for (Mg)
1	0.80	0.27	4.45	0.24	5.76
2	0.77	0.26	4.29	0.33	5.61
3	0.91[1]	—	4.44	0.36	5.71
4	0.91[1]	—	5.29	0.37	5.57
5	0.74	0.26	4.33	0.33	5.66
6	0.74	0.28	4.26	0.32	5.60

Source: Earle and Milner (1938).

[1] Also includes inorganic phosphorus.

designated as phosphatide phosphorus, was removed with petroleum ether. This value will probably vary somewhat with the amount of moisture in the meal at the time of extraction, since with increasing moisture level there is an increase in the amount of phosphatides removed with the hydrocarbon solvent.

Phospholipids

The phosphatides or phospholipids are fat-like substances which contain nitrogen and phosphorus. The phosphorus usually occurs as phosphoric acid or inositol in the molecule and the nitrogen as lecithin or cephalin. While the phospholipids are found in most oilseeds they are especially abundant in the soybean. The phosphatides are good emulsifying agents, soluble in alcohol and insoluble in acetone.

The soybean processors refer to the mixture of phospholipids, which are partly removed with the oil in solvent extraction processing, as "soybean lecithin." The lecithin is removed from the oil with a centrifuge after it has been hydrated at an elevated temperature with a small amount of water or steam. Prior to refining, the crude lecithin contains about 30% oil. After removing the oil with acetone it has been further characterized by Scholfield *et al.* (1948) in the Craig apparatus. They found that the defatted "soybean lecithin" consisted of approximately 29% lecithin, 31% cephalin, and 40% inositol-containing phosphilipids. Since the hexane used for extracting the oil removes only part of the phospholipids, we can assume that the fraction left in the meal is approximately the same composition as that which was removed.

Carter *et al.* (1958A,B) isolated and partially characterized, from the inositol-containing phospholipid fraction, a phytoglycolipid, which is described also as a phytosphingosine containing phosphoglycolipid. On hydrolysis they reported that this complex lipid contained phytosphingosine, fatty acids, phosphate, inositol, glucosamine, hexuronic acid, galactose, arabinose, and mannose.

Nucleic Acids

According to Di Carlo *et al.* (1955), whole soybeans contain 1.05% nucleic acid and the defatted soybean meal 1.30% in comparison to yeast in which nucleic acid ranges between 2.0% and 7.7%. They found that soybeans contain very little if any deoxyribonucleic acid (DNA). The composition of soy protein nucleic acid (PNA), terminology used by DiCarlo et al. (1955), as determined by chromatography and spectrophotometric techniques, was found to differ from the PNA isolated from yeasts and other sources. Table 3.15 shows the proportion of bases in yeast and soybean nucleic acids.

Mori *et al.* (1969) have reported that H-RNA in soybeans does not exist in the free state but as some particular component such as ribonucleoprotein.

MINOR ORGANIC CONSTITUENTS

Phenolic Acids

Phenolic acids have a wide distribution in the plant kingdom and although they usually occur in low concentration they have a significant role in soybean foods because of their possible effect on flavor of the soy flour and other products with which they are combined. Arai *et al.* (1966) extracted and identified a number of phenolic acids from hexane-defatted soy flour. They extracted 500 gm of defatted soyflour with 2 liters of 50% ethanol for 5 hrs at 80° C and found this solution to have a strong phenolic-like odor and flavor. On fractionation of the extract and use of paper chromatography, they found the solution to contain nine or more phenolic acids which were considered to have some influence on the flavor of the soy flour. They identified the following acids: syringic, vanillic, ferulic, gentisic, salicylic, *p*-coumaric and *p*-hydroxy-

TABLE 3.15

MOLAR PROPORTIONS OF BASES IN YEAST AND SOYBEAN RIBONUCLEIC ACID

	Yeast PNA		Soy PNA	
Base	Prepn A[1]	Prepn B[2]	Crude[3]	Purified[4]
Adenine	1.05	1.09	1.03	1.00
Guanine	1.19	1.19	1.29	1.00
Cytosine	0.83	0.77	0.88	0.92
Uracil	0.93	0.95	0.80	0.74

Source: Di Carlo *et al.* (1955).

Molar ratio of purines/pyrimidines:

[1] 1.27

[2] 1.32

[3] 1.37

[4] 1.41

benzoic acids. Of this group the principal component was syringic acid. They identified, also, two isomers of chlorogenic acid, presumably isochlorogenic and chlorogenic acid. They reported that the chlorogenic acids have sour, bitter, astringent, and phenolic-like flavors.

Rackis *et al.* (1970), in studies on soybean factors which cause gas formation in the intestines by the intestinal bacteria, reported that the phenolic acids, especially syringic and ferulic, are effective inhibitors *in vitro* and in the intestinal segments of dogs.

Other Organic Components

In addition to the isolation of phenolic compounds from dehulled soybeans, Fujimaki *et al.* (1969) made an extensive investigation of the minor organic components of the soybean with the objective of identifying the components responsible for the flavor of the bean and derived products. However, many of the products they identified occurred as the result of processing the beans for oil and protein or of the action of natural enzymes, such as lipoxidase, and they found it difficult to distinguish such components. They found, also, that many of the minor components were combined with the protein or other major components of the seed and thus were difficult to isolate and identify. These minor components will be discussed further in Chapter 10 under flavor problems.

SOLUBLE CARBOHYDRATES

The carbohydrates of the soybean have not been studied seriously as a potential source of food or feed. Their principal utilization is in animal feeds where they contribute some calories to the diet, especially for ruminants, since the latter make better use of the polysaccharides than monogastric animals.

The carbohydrates, like other components, have been shown to vary widely in the soybean; for example, O'Kelly and Gieger (1937) reported that the nitrogen free extract varied between 17.93% and 30.18%. The reported variations in the specific carbohydrates may arise partly in the difficulty of separation, purification, and analysis, and partly because of the influence of varietal and environmental factors in production. For example, when there is a change in the ratio of protein to oil, there are also changes in other components of the bean; usually, there is an inverse relationship between the oil and carbohydrates with the protein.

A crude extract of the soluble sugars can be made by refluxing the defatted soybean meal with 60–95% ethanol and filtering. For identifying specific sugars further purification of the extract is necessary.

The principal sugars of the soybean are the disaccharide sucrose, $C_{12}H_{22}O_{11}$, the trisaccharide raffinose, $C_{18}H_{32}O_{16}$, and the tetrasaccharide stachyose, $C_{24}H_{42}O_{21}$. A pentasaccharide verbascose, $C_{30}H_{52}O_{26}$, has been found in

very minor amounts. Glucose or other reducing sugars are present in green or immature beans in substantial amounts, but they disappear as the beans approach maturity and the occurrence of glucose in the mature soybean is questionable. The alcohol extract of defatted soybean meal has been reported to contain sucrose, raffinose, stachyose, fructose, galactose, rhamnose, arabinose, and glucuronic acid.

MacMasters *et al.* (1941) reviewed the early work on the carbohydrates of the soybean, and extended these investigations by determining the amount of total sugars, pentosans, and galactans and calculated total carbohydrates by difference for nine varieties of garden type soybeans at different stages of maturity. The total sugars, galactans, and pentosans were selected for study because preliminary data indicated they might be interrelated during growth. The stages investigated were precooking, cooking, postcooking, and maturity. A summary of their results is given in Table 3.16.

These results show that the average values for total carbohydrates (by difference) decreased from the immature precooking stage to maturity from 44.6 to 35.4%, the total sugars decreased from an average of 23.4 to 9.4%, and the reducing sugars decreased from an average of 7.4% to a value too low for analysis. The pentosans and galactans increased from 2.6 to 3.6% and 1.3 to 2.3%, respectively. While other workers have reported small amounts of starch in soybeans, MacMasters *et al.* (1941) were unable to identify starch by microscopic examination. Starch is frequently reported in immature beans but is seldom found in mature beans.

Cartter and Hopper (1942) determined the total sugars which was reported as sucrose, in field type beans for 10 varieties of soybeans, over a 5-yr period at 5 locations; the averages for the 10 varieties are shown in Table 3.1. The Illini variety had the highest average of 8.83% and the Mandarin a minimum average of 6.76%. The individual sugar values, however, range from a low of 2.76% for the Dunfield A variety to a high of 11.97% for the P.I. 54563-3 variety. The

TABLE 3.16

CARBOHYDRATES IN GARDEN TYPE SOYBEANS AT DIFFERENT STAGES OF MATURITY (VACUUM DRIED BASIS)

Stage of Maturity	Range of Total Carbohydrates (by Difference) (%)	Range of Total Sugars (%)	Range of Reducing Sugars (%)	Range of Pentosans (%)	Range of Galactans (%)
Precooking	38.4–51.3	16.5–31.7	5.8–9.7	2.4–3.1	1.2–1.6
Cooking	31.7–49.7	11.7–22.8	4.8–7.4	2.4–3.1	1.7–2.7
Postcooking	31.6–42.2	5.9–16.2	1.8–5.6	2.8–3.8	1.5–3.1
Mature	31.1–43.9	7.6–10.4	–	3.4–3.8	2.0–2.72

Source: MacMasters *et al.* (1941).

overall average for the 10 varieties was 7.97%. They stated that the greater number of analytical values varied less than 1% from the mean.

A relationship between the oil and carbohydrate content of oilseeds was pointed out by Garner *et al.* (1914) when they stated that "as a consequence of the physiological relationship of oil to carbohydrates, it appears that maximum oil production in the plants requires conditions of nutrition favorable to the accumulation of carbohydrates during the vegetative period and the transformation of carbohydrates into oil during the reproductive period." The results of Cartter and Hopper (1942) agree with this generalization in that the percentage of sugars increases and decreases with the percentage of oil and that when total sugar and oil changes in one direction, the protein content changes in the other.

Sucrose

Kraybill *et al.* (1937) isolated and identified sucrose from soybeans by two methods. Fat free flakes were extracted with 99% ethanol (temperature was not mentioned) and the alcohol extract was concentrated on a steam bath until sugar crystallized on the sides of the beaker. In a second method, an 80% ethanol extract of fat free flakes was concentrated before a fan to a thick syrup. Two liters of water were added and the solution treated with lead acetate; the precipitate was removed and the filtrate treated with barium hydroxide, and the excess lead and barium removed with sulfuric acid. The solution was shaken with ether to remove the acetic acid and then concentrated to a thick syrup. An equal volume of 99% alcohol was added causing the syrup to settle to the bottom. After stirring the syrup in the alcohol on the steam bath, the alcohol was decanted and the solution was left standing for several days for the formation of crystals of sucrose.

In another experiment, Kraybill *et al.* (1937) crystallized sucrose from an acetone extract of fat free flakes. The acetone solution was evaporated and the residue was extracted with ether to remove small amounts of fat; the residue was taken up in water and the sucrose precipitated by the addition of ethanol.

Raffinose

Raffinose is a nonreducing sugar without food value unless it has been hydrolyzed by strong acids into its components of galactose, glucose, and fructose. The raffinose can be hydrolyzed by enzymes two ways. Invertase will hydrolyze the sucrose part of the molecule to give melibiose and D-fructose. The enzyme emulsin, which contains an α-D-galactosidase as well as a β-glucosidase, can hydrolyze the melibiose residue to yield galactose and sucrose. Bottom yeasts, which contain both enzymes, can completely hydrolyze raffinose.

Stachyose and Verbascose

French *et al.* (1953) investigated the hydrolytic products of stachyose as a means of determining the type of linkage between the D-galactose and D-glucose

units in the molecule. They found that partial hydrolysis of stachyose by an almond emulsin preparation gave D-galactose and raffinose, sucrose and galactobiose. An acid hydrolysis of stachyose to D-fructose and manninotriose, followed by reduction of the manninotriose to manninotriitol and partial acid hydrolysis gives D-galactose, melibiitol, D-sorbitol, and galactobiose. A periodate oxidation of manninotriitol and manninotriose-1-phenylflavazole confirm the presence of a 1,6 linkage between the D-galactose and D-glucose units in stachyose. They report that stachyose is *O*-*a*-D-galactopyranosyl-(1→6)-*O*-*a*- D-galactopyranosyl-(1→6)-*O*-*a*-D-glucopyranosyl-*β*-D-fructofuranoside.

Kasai and Kawamura (1966) used dextran gel filtration for the isolation and purification of sucrose, raffinose, stachyose, and verbascose. They found that Sephadex G-15, which may be applied to compounds with molecular weights below 1500, was superior to carbon column chromatography, especially for isolating and purifying of stachyose. The Sephadex G-15 elutes first the highest molecular weight whereas the carbon column elutes the lowest molecular weight.

The sugars were extracted from both the defatted raw and autoclaved flakes. The treatment in the autoclave was at 120° C for 10 min at a moisture level of 20%. The melting points and specific rotations were determined on the sugars and their acetate derivatives, and reported in Table 3.17.

Kawamura and associates (1967) examined the sugars of the whole soybean and of the defatted seed parts for 6 U.S. and 3 Japanese varieties by quantitative paper chromatography. He estimated the maximum variation by his method as ± 10%, but did not have a standard for verbascose. The total sugars were determined by anthrone colorimetry.

The sugars were extracted from the defatted parts of the bean by refluxing with 80% ethanol for 1 hr, filtering, and washing the residue with cold water until the washings gave a negative sugar reaction with anthrone. The combined extracts and washings were concentrated under reduced pressure below 40° C. The average results for the 6 U.S. and 3 Japanese varieties are in Table 3.18.

The author states that the arabinose reported in the hull may not be free but could have come from the water-soluble polysaccharides which were liberated in the course of the isolation of the other components. These data indicate that the Japanese soybeans may be somewhat higher in sugars than U.S. soybeans.

Kawamura's results on a dry basis for U.S. whole soybeans are sucrose 4.5%, raffinose 1.1%, stachyose 3.7%, and traces of arabinose and glucose for a total of 9.3% sugars. For the Japanese beans the values are sucrose 5.7%, raffinose 1.1%, stachyose 4.1%, and traces of arabinose and glucose for a total of 10.9% sugars.

If we assume 20% oil for the whole bean, then the defatted soybean flakes will contain approximately 11.6% total soluble sugars and the Japanese beans 13.6% total soluble sugars. In earlier work and by a different method, Cartter and Hopper (1942), working on 10 varieties of well composited U.S. soybeans determined total sugars as sucrose and found 7.97% for whole soybeans. Assum-

TABLE 3.17
MELTING POINTS AND SPECIFIC ROTATIONS OF SUGARS AND ACETATES OF SUGAR FROM RAW AND AUTOCLAVED SOYBEAN FLAKES

	Melting Point, °C		$[\alpha_D]$	
	Found	Literature	Found	Literature
Sugars				
Sucrose	176–180	184–185	+ 66.7/24° (H_2O)	+ 66.5° (H_2O)
Raffinose	118–120	118–120	+123.4/20° (H_2O)	+123.1° (H_2O)
Stachyose	170–172	170	+146.2/20° (H_2O)	+146.3° (H_2O)
Acetates of Sugars				
Sucrose octaacetate	87	87	+ 59.0/20° ($CHCl_3$)	+ 59.6° ($CHCl_3$)
Raffinose hendecaacetate	98–101	99–101	+ 97.4/16° (EtOH)	+ 92° (EtOH)
Stachyose tetradecaacetate	94–95	95–96	+120.1/16° (EtOH)	+120.2° (EtOH

Source: Kasai and Kawamura (1966).

TABLE 3.18

SUGARS IN SEED PARTS OF U.S. AND JAPANESE SOYBEANS

	Whole Soybeans (%)	Defatted Cotyledons (%)	Defatted Hypocotyl (%)	Hull (%)
	U.S. Soybeans: Avg 6 Varieties			
Sucrose	4.5	6.2	6.0	0.58
Raffinose	1.1	1.4	1.7	0.11
Stachyose	3.7	5.2	8.4	0.39
Verbascose				
Arabinose	0.002			0.023
Glucose	0.005			0.06
	Japanese Soybeans: Avg 3 Varieties			
Sucrose	5.7	7.4	9.6	0.64
Raffinose	1.1	1.4	2.1	0.16
Stachyose	4.1	5.4	6.7	0.45
Verbascose				
Arabinose	0.001			0.015
Glucose	0.007			0.04

Source: Kawamura (1967).

ing 20% oil in the soybeans this would be equivalent to 9.96% of total sugars in the defatted flakes, a value which agrees quite well with the 9.3% found by Kawamura.

INSOLUBLE CARBOHYDRATES OF COTYLEDONS

A limited amount of work on the polysaccharides of the cotyledons has been reported by Aspinall *et al.* (1967). Starting with defatted meal (they did not mention the presence or absence of the seed coat) the soluble sugars were extracted with boiling ethanol-water (4:1) and the protein removed with dilute alkali. However, they emphasized that they experienced difficulty in eliminating all of the protein.

The major part of the polysaccharides was isolated from the deproteinized meal by extraction with ammonium oxalate or, preferably, with ethylenediaminetetraacetic acid, disodium salt. Some additional polysaccharides were extracted with alkali although the latter fraction was not further examined. The fraction obtained by extraction with the oxalate contained a mixture of acid polysaccharides and arabinogalactan. Further extraction with water gave a mixture of arabinan and arabinogalactan.

In another experiment, they extracted defatted meal with phenol-acetic acid-water (1:1:1) and then with EDTA to give the same components as extracting the deproteinized meal with ammonium oxalate. The acidic polysaccharides extracted from the cotyledons were said to possess structures of

extreme complexity. Their results indicated that these polysaccharides may be regarded as belonging to the pectic group of substances.

SEED COAT

The total amount of seed coat or hull which enters the U.S. soybean processing plants annually, at the present level of processing, is about 1.6 million short tons. The hulls, which are the least valuable part of the seed, account for about 1/2 of the 6% of fiber which is present in the undehulled, defatted meal. Since fiber is usually held to a minimum in poultry feeds, most of the hulls are diverted into ruminant feeds and some are used as a carrier for vitamins. Although the nutritional value (Chap. 7) of the hulls is low, there has not been a serious effort made to upgrade this factor.

The percentage of hull varies somewhat with the size of the seed, the larger the seed the lower the proportion of hull. The percentage of hulls and the seed size, the size being measured by the weight in grams per 100 seeds, were determined for several varieties of soybeans by Cartter and Hopper (1942) and are reported in Table 3.19. The smallest seed in the group is the Peking variety, which has 12.98% hull and a seed weight of 6.29 gm, whereas the T-117 has 7.4% hull and a seed weight of 15.37 gm. The average hull for the 10 varieties is 8.28%. However, when the Peking, which is a black-seeded variety, is eliminated the average is 7.75%.

Kawamura and associates (1967) measured the relative amounts of hull, hypocotyl and cotyledon for 6 U.S. and 3 Japanese varieties and also determined

TABLE 3.19

PERCENTAGE OF HULLS FOR 10 VARIETIES AND STRAINS GROWN AT ONE LOCATION AND WEIGHT OF 100 SOYBEANS (MOISTURE EQUILIBRATED AT 70° F. AND 18% RELATIVE HUMIDITY)

Variety	Hull (%)	Seed Size Wt/100 (Gm)
Mandarin	8.49	14.88
Mukden	7.51	14.43
Dunfield A	7.71	14.47
Dunfield B	7.61	15.21
Illini	8.14	13.52
Manchu	7.36	14.79
Scioto	7.32	14.79
T-117	7.40	15.37
Pekin	12.98	6.92
P.I. 54563-3	8.25	12.82
Avg	8.28	13.6
Avg without Pekin	7.75	14.4

Source: Cartter and Hopper (1942).

the crude protein, fat, nitrogen free extract, plus fiber and ash in each part; his results are in Table 3.20.

The hull of the mature bean is hard, water resistant, and protects the cotyledons and hypocotyl or germ from damage which may be caused by weathering, harvesting, insects, and transportation. The dark scar on the seed is called the hilum, and at one end of the hilum is the micropyle, or small opening in the seed coat, which under favorable conditions will permit the entrance of moisture. The hull is loosely attached to the cotyledons and when the dry mature seed is cracked, as in oil mill processing, the hull is detached fairly readily and separated from the cotyledons by aspiration.

The color of the hull may be different from that of the cotyledons; it may be black, brown, blue, mottled, green, or various shades of yellow. The present trend in plant breeding is to develop varieties with a light yellow seed coat. A white coated seed would be the most desirable. In processing soybeans for oil it is not practical to attempt a complete removal of the hulls; thus, soybeans having a dark colored seed coat will leave dark specks in the meal, an undesirable factor when used in food products.

Chemical Composition

The composition of soybean hulls is difficult to determine and analytical results reported from different laboratories often do not agree. Nelson *et al.* (1950) determined the composition of a large number of crop seed hulls

TABLE 3.20

AVERAGE COMPOSITION OF SOYBEAN SEED PARTS OF 6 U.S. AND 3 JAPANESE VARIETIES

	Whole Soybeans (%)	Full-fat Cotyledons (%)	Full-fat Hypocotyl (%)	Hull (%)
U.S. Soybeans: Avg 6 Varieties				
Crude protein	40.4	43.4	40.8	9.0
Crude fat	22.3	24.3	12.0	0.9
N-free extract + fiber	31.9	27.4	42.7	86.2
Ash	4.9	5.0	4.5	4.0
Japanese Soybeans: Avg 3 Varieties				
Crude protein	39.2	41.7	40.7	8.6
Crude fat	18.4	20.0	11.2	1.3
N-free extract + fiber	37.4	33.3	44.0	85.6
Ash	5.0	5.0	4.2	4.5

Source: Kawamura (1967).

Average yield for the 3 parts was cotyledons 90.3%, hypocotyl 2.42%, and hull 7.25%.

(including soybean hulls), nut shells, and fruit pits. Table 3.21 gives the analysis of raw and cooked soybean hulls as reported by Nelson *et al.* The principal components of the hull are cellulosic type materials 49.3%, pentosans 22.6%, lignin 4.5%, ash 5.7%, and nitrogen 1.6%.

Whistler and Saarnio (1957) analyzed soybean hulls and found that acetone-extracted hulls contained 64% alpha cellulose, 16% hemicellulose extractable with an alkaline solution, and 8% lignin. One of the celluloses was a galactomannan which was extractable with water at 40° C in a 2% yield. They found that the galactomannan has a D-galactose unit to a D-mannose unit in the ratio of 2:3 which they state compares with guaran which has an approximate ratio of 1:2, reported for the fractionated guaran. The soybean galactomannan yielded on acid hydrolysis only D-galactose and D-mannose which were separated chromatographically and obtained in crystalline form.

In another investigation, Sanella and Whistler (1962) reported a partial analysis of soybean hulls which gave them alpha cellulose 49.8%, lignin 7.8%, hemicellulose A 10.6%, hemicellulose B 6.0%, crude protein 13.6%, and ash 4.9%. The hemicellulose B was an alkali-soluble hemicellulose which was not precipitated with acid. This fraction contained an acid polysaccharide as a major component and on purification and hydrolysis they identified D-xylose, L-arabinose, D-glucose and a galactomannan occurring in molar ratios of 14:1:3:3.

Aspinall *et al.* (1967) also reported on the polysaccharides of the seed coat. They found four general types of polysaccharides, namely galactomannans, a group of related polysaccharides of the pectic type, xylan, and cellulose. In a further study of the above fractions an estimation was made of the various hydrolytic products. However, a quantitative evaluation of the hydrolytic prod-

TABLE 3.21

PARTIAL CHEMICAL ANALYSIS[1] OF SOYBEAN HULLS (OVEN DRY BASIS)

	Raw Inventory No. CD 12701 (%)	Cooked Inventory No. CD 12702 (%)
Ash content	5.7	5.6
Solubility in:		
Alcohol-benzene	4.6	4.9
Hot water	17.1	16.9
1% NaOH solution	45.6	46.0
Lignin	4.5	5.7
Pentosans	22.6	22.9

[1] Analysis supplied by E. G. Helman, Northern Regional Research Laboratory, USDA-ARS, Peoria, Ill.

ucts is complicated by the fact that the same sugars may be derived on hydrolysis of more than one type of polysaccharide. For example D-galactose and D-glucose are important constituents of the galactomannans and hemicellulose, respectively; also, both sugars are formed as hydrolysis products from acidic polysaccharides. Digestion of the hulls with cold 72% sulfuric acid followed by hydrolysis with dilute acid indicated the formation of 72% reducing sugars.

From their work, Aspinall *et al.* (1967) estimated the hulls contain 9–11% glactomannans, 10–12% acidic polysaccharides, 9–10% xylan hemicellulose, and about 40% cellulose. They found the hulls to contain about 11% protein and peptides and they state that the remaining material, not specifically accounted for, is probably lignin.

Amino Acids

The soybean seed coat contains 1.53–2.0% nitrogen. Although the source of the nitrogen has not been completely identified it is the equivalent of 10–12% protein. Rackis *et al.* (1961) determined the amino acids in the seed coat, along with the amino acids in the meal and protein fraction of the meal. The results reported in Table 3.7 show that most of the essential and related amino acids in the seed coat are in much lower concentration than in the meal, particularly phenylalanine, arginine, methionine, isoleucine, and leucine. However, lysine is slightly higher and tyrosine is 20% higher than in the meal. The seed coat contains more than double the amount of glycine found in the meal and 7.56% hydroxyproline, whereas the dehulled meal and residue from protein isolation do not contain hydroxyproline. At periods of 72 and 90 hrs of hydrolysis there was extensive destruction of hydroxyproline and a moderate destruction of proline; thus, the values were obtained by extrapolating to zero.

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CHAPTER 4

W. J. Wolf

Purification and Properties of the Proteins

INTRODUCTION

Work done in the past 20 yr shows that proteins prepared by earlier workers were mixtures and that several of the proteins undergo complex reactions. At least seven soybean proteins now appear to be made up of subunits, which may be disrupted under a variety of conditions. Because of their subunit structure the major soybean proteins have molecular weights ranging from about 200,000 to 600,000. In the native state, these large molecules can form still higher particle sizes either through association-dissociation reactions or by forming disulfide-linked polymers. Because of this complexity it is necessary to fractionate soybean proteins before detailed studies are made on them.

This chapter describes progress made in the isolation and characterization of soybean proteins in the past two decades and points out where further work is needed. Coverage is mainly on "storage proteins," but biologically active proteins, such as trypsin inhibitors and hemagglutinins that are also discussed in Chap. 6, are included for completeness. A detailed summary of the chemistry of soybean proteins up to 1948–1949 was compiled by Circle (1950). Brief reviews since 1948–1949 are also available (Wolf and Smith 1961; Bain *et al.* 1961; Wolf 1969A, 1970A).

NOMENCLATURE

At present there is no nomenclature system generally accepted for soybean proteins. However, this problem is currently under study and a detailed discussion of past terminology, as well as proposals under consideration, can be found elsewhere (Wolf 1969B). Some of the names being considered have recently been introduced (Catsimpoolas 1969A; Catsimpoolas and Ekenstam 1969), but a final decision on terminology has not been reached.

The nomenclature system based on approximate sedimentation coefficients as introduced by Naismith (1955) has been used extensively in the past decade. Because of its adoption by many workers this terminology, as exemplified in Fig. 4.1 is used here. Figure 4.1 also illustrates a shortcoming of the ultracentrifuge terminology: Sedimentation properties of soybean proteins depend on conditions of buffer composition, pH, and other factors. For example, a portion of the 7S fraction observed at pH 7.6, 0.5 ionic strength, dimerizes at 0.1 ionic strength to form a 9S peak, as indicated at the bottom of Fig. 4.1.