Conclusions

1. On the basis of adsorbent activity the acid activated clays are generally 1½ to 2 times more effective as bleaching agents than the natural earths.
2. The activity of an adsorbent in bleaching a vegetable oil is at a maximum at some particular temperature.
3. The acid-activated clays that were tested had a temperature of maximum activity in the range of 100 to 106°C.
4. The natural earths that were tested had a temperature of maximum activity in the range of 118 to 132°C.
5. The three materials tested that were high in silica content had a temperature of maximum activity in the range of 180 to 250°C., which is high for effective use in the edible oil industry.

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The Study of 20 Varieties of Soybeans With Respect to Quantity and Quality of Oil, Isolated Protein, and Nutritional Value of the Meal

O. H. ALDERKS, Soybean Research Council, Technical Division, The Buckeye Cotton Oil Company,*
M. A. & R. Building, Ivorydale 17, Ohio

Summary

TWENTY samples of soybeans, representing the most promising strains now being grown at several stations were selected and prepared by J. L. Cartter and colleagues of the U. S. Regional Soybean Laboratory. Ten samples represented strains grown in the north central states and ten in the southern states. They were composited samples of the uniform soybean variety tests and therefore location and soil differences cancelled out, giving a good comparison of germ plasm.

Studies of these soybeans included the following:

1. Yield and quality of the oil with respect to flavor reversion.
2. Yield and quality of isolated protein for industrial usage.
3. Quantity and quality of protein for nutritional purposes as indicated by: a) Amino acid composition of solvent extracted raw and toasted flakes; b) availability of amino acids based on in-vitro enzyme digestion tests.

The following is a summary of the results:

1. Samples analyzed for oil varied from 17.41% to 22.9% dry basis, equivalent to a difference of 98.8 lb. per ton of 10% moisture beans. Roanoke contained the highest per cent.
2. Samples analyzed for protein varied from 39% to 44%, dry basis, equivalent to a difference of 91.8 lb. per ton of 10% moisture beans.
3. Three strains grown at Lafayette, Ind., Ames, Ia., Urbana, Ill., and Columbus, O., varied as much as 1.79% oil and 3.1% protein, dry basis.
4. The fatty acid composition varied considerably. The triple unsaturated acids varied as follows:
   Per cent linolenic acid from 6.16% to 8.45%
   Per cent arachidonic acid from .01% to .09%.
   If these are important in the development of "flavor reversion," one would expect no differences in the oils in this respect.
5. The quality and flavor stability of each solvent extracted oil from the 20 strains was top No. 1 grade. The oils were equal in flavor stability to the best quality commercial oils. All oils showed "flavor reversion" after aging in open new tin cans at 140°F. for several days.
6. The average yield of all 20 strains of alkali extracted, purified, isolated protein was 36.1% (DB). The yields varied widely from 33.1% to 42.7% (DB). Roanoke and Arksoy 2913 gave the highest yields of industrial protein.
7. No significant differences were found between the amounts of any one of the "essential" amino acids contained in the 20 soybean strains. There is no indication in this work that a strain might be selected and grown for the production of soybean meal with a superior protein. The "toasted" flakes contained less lysine than the hexane extracted, desolventized, raw flakes (average drop from 6.60% to 6.28%). There was no effect on the other "essential" amino acids.
8. In-vitro enzyme digestion followed by microbiological assay was applied to methionine, lysine, and tryptophane. When the results are considered in the light of the accuracy of these new methods, it appears certain that there are no significant differences between the varieties in available tryptophane, but there is some possibility of significant differences in the availability of methionine and lysine.

This work was planned with J. L. Cartter of the U. S. Regional Soybean Laboratory and R. T. Milner of the Northern Regional Laboratory to determine whether the genetic composition of various soybean strains differed in regard to their ability to produce oil of good quality and whether the oils from these various strains might differ in flavor stability for any reason. Also we desired to determine if by agronomic selection and development of soybean varieties it would be possible to reduce the linolenic acid content in soybean oil.

Some investigators working on the soybean oil flavor reversion problem have attributed flavor reversion to result from oxidation products of the more unsaturated portion of the oil. It has been suggested also that various minor constituents in the oil possibly affected flavor stability by acting as catalysts to promote a certain type of oxidation. Cottonseed, peanut, sesame, and sunflower seed oils contain no linolenic or triple unsaturated fatty acids, and flavor reversion of the soybean oil type is not encountered with these oils. If linolenic acid could be eliminated

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from the composition of soybean oil perhaps the flavor reversion problem would be eliminated as well. In addition to quality of oil the strains of soybeans were examined for:

1. Quantity of oil in the soybeans.
2. Quantity and quality of pure proteins obtainable from the hexane extracted flakes suitable for industrial usage.
3. Quantity and quality of protein for nutritional purposes as indicated by: a) Amino acid composition of raw and toasted flakes; b) availability of amino acids based on in-vitro enzyme digestion tests.

The 20 different strains of soybeans investigated in this work represented the most promising strains that are now being grown and investigated at the various stations.

Three strains (Lincoln, Richland, A-4-107-12) were composites prepared by mixing equal quantities of seed from each of four locations in the corn belt (Lafayette, Ind., Urbana, Ill., Ames, Ia., and Columbus, O.) so that the resulting lots would reflect a general average of growing conditions and would give a good comparison between varieties. Seven other strains from the north central states and ten strains from the southern states were made up of carefully composited samples from the uniform soybean variety tests. The samples were prepared in this manner so that location and soil differences would cancel out as nearly as possible, thus giving a good comparison of germ plasm. A description of each strain obtained from J. L. Cartter is attached in the appendix.

1. YIELD AND QUALITY OF SOYBEAN OILS FROM 20 VARIETIES OF SOYBEANS

Representative samples of the 20 strains of soybeans were analyzed in duplicate for % oil, % NH_3, and % H_2O. The data are reported in Table I and show a difference in oil content ranging from 17.41% to 22.90% (DB). The difference in protein content

TABLE I

Soybean Analysis
(Samples analyzed in duplicate as indicated)

Strain	% Oil (DB)	% Protein (DB)	% H_2O
1. Lincoln Composite	20.64, 20.58	41.92, 42.00	9.4, 9.4
2. Richland Composite	20.25, 20.20	41.38, 41.31	9.1, 9.2
3. A4-107-12	20.58, 20.25	42.44, 42.41	8.7, 8.7
4. A3K-884			
5. H5			
6. C-463			
7. Chief	Samples lost due to accident in laboratory		
8. Earlyana			
9. A3-176			
10. Lincoln			
11. S-100	19.89, 19.84	42.69, 42.56	8.5
12. Gibson			
13. Ogden	21.10, 21.10	41.13, 41.53	7.8
14. Arksoy 2913	20.09, 19.87	43.53, 43.31	7.9
15. N-44-92			
16. N44-774	19.76, 19.92	41.63, 41.44	7.4
17. Roanoke	22.62, 22.90	39.88, 39.81	7.6
18. C.N.S.			
19. Acadian	19.64, 20.08	39.47, 39.31	9.1
20. Mamloxi	17.41, 17.63	44.19, 44.41	8.4

TABLE I(A)

Lincoln			
A. Lafayette, Ind.	20.48, 20.33	42.10, 42.38	9.9, 9.5
B. Ames, Ia.	20.77, 20.88	42.06, 41.88	9.5, 9.5
C. Urbana, Ill.	21.44, 21.44	40.56, 40.25	9.3, 9.5
D. Columbus, O.	19.85, 19.65	43.00, 43.31	8.8, 8.9
Richland			
A. Lafayette	20.59, 20.46	41.53, 41.56	9.2, 9.1
B. Ames	20.22, 20.15	40.69, 40.56	9.0, 9.2
C. Urbana	20.76, 20.81	40.19, 40.06	8.7, 8.7
D. Columbus	19.47, 19.32	43.00, 43.25	9.6, 9.7
A4-107-12			
A. Lafayette	21.03, 20.89	40.94, 40.69	8.0, 8.1
B. Ames	21.24, 21.26	42.19, 42.66	8.9, 9.0
C. Urbana	20.48, 20.44	42.94, 42.81	9.2, 9.0
D. Columbus	19.57, 19.58	43.75, 43.44	8.8, 8.6

TABLE II

Analysis of Hexane Extracted Flakes

Strain of Soybeans	% H_2O	% Oil* (DB)	% NH_3 (DB)	% Prot. (DB)
1. Lincoln	10.2	.67	10.58	54.38
2. Richland	9.8	.73	10.29	52.88
3. A4-107-12	10.5	.94	10.70	55.00
4. A3K-884	10.1	.85	10.51	54.00
5. H-5	10.5	.83	10.70	55.00
6. C-463	9.3	.82	10.62	54.56
7. Chief	10.3	.83	10.65	54.75
8. Earlyana	9.6	.66	10.76	55.31
9. A-3-176	10.4	.74	10.66	54.81
10. Lincoln	9.4	.68	10.76	55.31
11. S-100	10.4	.49	10.88	55.94
12. Gibson	9.4	.57	10.65	54.75
13. Ogden	9.5	.49	10.55	54.25
14. Arksoy 2913	10.0	.53	11.26	57.88
15. N-44-92	10.1	.49	10.73	55.13
16. N-44-774	9.7	.53	10.83	55.69
17. Roanoke	9.6	.49	10.56	54.28
18. C.N.S.	11.3	.49	11.31	58.13
19. Acadian	10.0	.60	10.78	55.41
20. Mamloxi	10.2	.58	10.89	55.97
Blank	9.9	.42	10.77	55.38

* Skellysolve F.

of the strains varied from 39% to 44.0% (DB). These are important differences to soybean processors, being equivalent to a maximum difference of 3 lb. oil per bushel of soybeans. The variety Roanoke contained the most oil, namely, 22.76% (DB). In Table I-A the individual analyses are shown for Lincoln, Richland, and A4-107-12 strains grown at Lafayette, Ind., Ames, Ia., Urbana, Ill., and Columbus, O. The same variety grown at the four locations in the corn belt shows a maximum difference in oil content from 19.65% to 22.4% (DB) and in protein from 40% to 43% (DB).

Pilot Plant Processing for Oil

The half-bushel lots of soybeans as received in cotton bags were stored together in steel drums for a period of about a week to attain moisture equilibrium. Each sample of beans was hulled by means of a laboratory bar huller and about 5% of hulls (based on bean weight) removed by air elutriation. The hulled, cracked, unheated beans (70°F.) were flaked, through a one-pass laboratory flaking roll, to an average flake thickness of .005". The flakes, at 85°F., were placed in an extraction basket equipped with a flat bottom which could be closed to hold the solvent on the flakes. The flakes were given three washes with hexane at 130-135°F. as follows:

1st wash—10 gal. hexane—holding time on flks.—15 min.—drain time 5 min.
2nd wash— 9 gal. hexane—holding time on flks.—15 min.—drain time 5 min.
3rd wash—10 gal. hexane—holding time on flks.— 3 min.—drain time 5 min.

The hexane washes were concentrated in a forced circulation still and finished under vacuum in glass. The maximum oil finishing temperature was 200°F. These samples of solvent extracted crude oil were examined for flavor stability as described below.

Fatty Acid Composition of Oils

Samples of freshly extracted oil, from 13 strains of soybeans, were analyzed for fatty acid composition, using the methods being investigated by the committee for the spectrophotometric analysis of Fats and Oils (1).

These data are shown in Table III, and indicate the following variations:

1. Per cent linoleic acid, range, 49.26%-58.62%.
2. Per cent linolenic acid, range, 6.16%-8.45%.
3. Per cent arachidonic acid, range, .01%-.09%.

TABLE III

Analyses of Hexane Extracted Crude Soybean Oils

Strain	No. 1 Lincoln Comp.	No. 2 Richland Comp.	No. 3 A4-107-12 Comp.	No. 5 H-5	No. 6 C-463	No. 11 S-100	No. 12 Gibson	No. 13 Ogden	No. 14 Arksoy 2913	No. 16 N44 774	No. 17 Roanoke	No. 19 Acadian	No.20 Mamloxi
% Apparent Linoleic Acid	55.79	49.72	49.26	51.19	51.16	51.49	53.20	56.51	56.16	52.74	56.73	56.60	58.62
% Apparent Linolenic Acid	7.70	7.25	7.07	8.45	7.18	6.49	7.23	6.96	6.16	7.21	7.14	7.33	6.91
% Apparent Arachidonic Acid	0.04	0.03	0.03	0.03	0.03	0.09	0.02	0.01	0.08	0.03	0.03	0.03	0.02
% Apparent Conjugated Diene	0.13	0.14	0.13	0.15	0.15	0.23	0.16	0.17	0.21	0.16	0.16	0.16	0.17
% Apparent Conjugated Triene	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
% Apparent Conjugated Tetraene	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
% Apparent Oleic Acid (Calc.)	15.4	20.5	22.1	19.2	18.03	20.2	16.6	14.8	15.3	19.1	14.2	15.2	11.4
% Apparent Saturated Acids—Calc. Assuming 95.7% TFA	16.6	18.1	17.1	16.7	19.2	17.2	18.3	17.3	17.8	16.5	17.4	16.4	18.6
I.V.	136.3	128.7	128.8	133.5	130.7	129.9	131.4	135.0	133.0	132.8	135.4	136.6	135.7
S.V.	189.5	188.3	184.9	191.2	192.3	188.1	191.2	191.1	189.1	189.2	190.7	188.3	189.8

4. Per cent oleic acid, range, 11.4%-22.1%.
5. Per cent saturated acids, range, 16.4%-19.2%.
6. Per cent conjugated diene, range, .13%-.23%.
7. Iodine value, range, 128.7-136.6.
8. Saponification value, range, 184.9-192.3.

These data suggest that from a fatty acid composition point of view one would expect little difference in the quality of the oil. If the linolenin content of soybean oil is an important factor in its flavor stability, then one would certainly not expect any difference in the flavor stability of these oils, since the per cent linolenic acid varied only from 6.16 to 8.45%.

Edibility Tests of Oil from the Twenty Strains of Soybeans

1. *Refining and Bleaching of Oils.* The hexane extracted crude soybean oils were refined in a 9-lb. refining kettle at low temperature with 10° Bé. caustic with results as shown in Table IV. The exact refining, bleaching, hydrogenation, deodorization, and flavor panel techniques used in this work are those described and illustrated in detail by J. H. Sanders (2). The FFA's of the extracted oils were low, varying from .3 to .9% and the refining losses were uniformly low with three exceptions. The refined oils were bleached with 3% earth. The bleach colors of these oils were remarkably uniform and low, varying from 1.1 to 1.5 red with two exceptions, viz., 1.7 and 2.3 red.

2. *Organoleptic Tests for Flavor.* All of the solvent extracted refined and bleached oils from the 20 strains of soybeans were tested for flavor stability, using an organoleptic flavor panel of several men with much experience in this type of work. In most cases both the unhydrogenated and hydrogenated oils were examined with results as shown in Table V. The unhydrogenated oils show exceptional uniformity of flavor quality. The aged flavors were obtained after exposure in new, open tin cans at 140°F. for two days. The hydrogenated (range 75 ± 5 IV) and deodorized oils had remarkably uniform low colors varying from .1 to .3 red. These oils were flavored fresh and after aging several days in new, open tin cans at 140°F.

The flavor results show that each of the oils was a No. 1 grade oil. The quality was uniformly top grade but not better in flavor stability and quality than normal No. 1 grade commercial oil. Sample No. 21 gives comparative flavor results of a commercial oil produced from prime yellow soybeans. Three samples of oil were aged at 90°F. for five months and showed fair flavors after this exposure (Table V). All of the oils showed flavor reversion after aging. If there were differences in quality and flavor stability among these oils, the present organoleptic tests as developed in this laboratory are not sufficiently accurate to show these slight differences in single tests.

TABLE IV

Refining and Bleaching Results of Oils

	Crude FFA %	Refining Results			Color	FFA %	Bleach Color (3% Earth)	% Trans. 660-670
		Lye %/°Bé	Silicate %	Loss %				
1. Lincoln Comp.	0.6	5.0 /10	0.5	4.1	50/8.5	....	.....	.....
2. Richland Comp.	0.6	5.0 /10	0.5	3.7	50/10.3	....	.....	.....
3. A4-107-12 Comp.	0.7	5.0 /10	0.5	4.1	50/8.8	....	.....	.....
4. A3K-884	0.3	4.55/10	0.5	2.4	70/8.1	.03	14/1.1	100
5. H-5	0.3	4.7 /10	0.5	3.0	25/8.4	.03	11/1.4	.....
6. C-463-Comp. No. 1	0.3	4.7 /10	0.5	4.2	35/6.4	.035	12/1.5	100
7. Chief	0.5	4.85/10	0.5	2.8	50/8.2	....	20/1.1	100
8. Earlyana	0.5	4.85/10	0.5	2.2	70/8.7	.03	15/1.5	99
9. A3-176	0.3	4.55/10	0.5	3.0	70/7.1	.03	15/1.3	100
10. Lincoln	0.9	5.45/10	0.5	6.9	70/7.5	.03	14/1.2	100
11. S-100	0.5	4.85/10	0.5	4.8	70/9.6	.04	45/2.3	100
12. Gibson	0.45	4.9 /10	0.5	3.8	.....	.03	12/1.5	.....
13. Ogden	0.4	4.8 /10	0.5	4.8	35/6.7	.025	13/1.5	78
14. Arksoy 2913	0.5	4.85/10	0.5	3.6	50/8.6	....	20/1.3	100
15. N44-92	0.5	4.85/10	0.5	3.0	50/7.1	.03	14/1.3	99
16. N44-774	0.4	4.8 /10	0.5	3.3	35/8.0	.055	13/1.5	79
17. Roanoke	0.3	4.7 /10	0.5	5.0	35/6.2	.035	11/1.4	89
18. C.N.S.	0.6	5.0 /10	0.5	9.7	50/6.5	.03	13/1.2	98
19. Acadian	0.5	4.9 /10	0.5	1.9	30/7.0	.035	12/1.5	.....
20. Mamloxi	0.6	5.0 /10	0.5	3.5	30/7.0	.03	12/1.5	.....
21. Blank (Yellow Midwestern)	0.6	5.0 /10	0.5	10.2	70/8.5	.04	30/1.7	100

TABLE V

Edibility Test Results on Oils Extracted From 20 Strains of Soybeans

	Unhardened Oil—Deodorized				Hardened Oil—Undeodorized			
	Color	% FFA	Fresh Flavor	Aged Flavor, 2 days	Refractive Index	IV	Color	% FFA
1. Lincoln Comp	.....	....	.....	.....	47.7	.....	.....	....
2. Richland Comp	.....	....	.....	.....	47.7	.....	.....	....
3. A4-107-12 Comp	.....	....	.....	.....	47.7	.....	.....	....
4. A3K-884	4/0.5	.02	Fair, Fair+, Nutty	Fair	49.0	79.4	2/0.4	.05
5. H-5	.....	....	.....	.....	47.6	70.5	2/0.3	.04
6. C-463 Comp. No. 1	.....	....	.....	.....	48.0	75.0	2/0.2	.03
7. Chief	2/0.3	.02	Fair+	Fair, Fair—	48.1	.....	3/0.4	.045
8. Earlyana	3/0.5	.02	Fair, Beany	Fair, Fair—	49.0	81.1	3/0.3	.05
9. A3-176	3/0.5	.02	Fair, Fair+, Nutty	Fair	49.0	80.5	3/0.3	.045
10. Lincoln	4/0.5	.025	Fair, Fair+, Nutty	Fair	48.9	81.1	3/0.3	.05
11. S-100	3/0.3	.02	Fair, Fair+	Fair	48.0	75.8	3/0.2	.05
12. Gibson	.....	....	.....	.....	47.7	72.7	2/0.3	.035
13. Ogden	.....	....	.....	.....	48.0	74.3	2/0.2	.03
14. Arksoy 2913	2/0.2	.02	Fair+	Fair, Fair—	48.0	76.9	2/0.3	.06
15. N44-92	4/0.5	.025	Fair, Beany	Fair—, Beany	48.1	81.3	4/0.4	.045
16. N44-774	.....	....	.....	.....	48.0	76.5	2/0.2	.03
17. Roanoke	.....	....	.....	.....	48.1	74.3	2/0.2	.03
18. C.N.S	4/0.4	.03	Fair, Beany	Fair—, Beany	47.8	72.8	4/0.3	.05
19. Acadian	.....	....	.....	.....	48.0	75.3	2/0.3	.045
20. Mamloxi	.....	....	.....	.....	48.0	74 4	2/0.3	.035
21. Blank	3/0.4	.02	Fair	Fair, Fair—, Beany	48.0	.....	3/0.3	.05

	Hardened Oil—Deodorized					
	Color	% FFA	Fresh Flavor	Aged Flavor		
				3 days	5 days	7 days
1. Lincoln Comp	1/0.2	.02	Fair+	Fair (1) (sl. buttery)	.....	.....
2. Richland Comp	1/0.3	.02	Fair+	Fair (2) (sl. buttery)	.....	.....
3. A4-107-12 Comp	1/0.3	.02	Fair+	Fair (3) (sl. Mariny)	.....	.....
4. A3K-884	2/0.3	.02	Fair+	Fair	Fair—	.....
5. H-5	1/0.2	.015	Fair+	Fair	.....	.....
6. C-463 Comp. No. 1	1/0.1	.015	Good	Fair+	Fair	Fair—, Pumpkin
7. Chief	2/0.3	.02	Good—, Fair+	Fair—(Pumpkin)	.....	Fair—
8. Earlyana	2/0.2	.02	Fair+	Fair	Fair, Fair—	.....
9. A3-176	2/0.2	.02	Fair+	Fair, Fair+	Fair, Fair—	.....
10. Lincoln	2/0.2	.02	Fair+	Fair	Fair—	.....
11. S-100	1/0.2	.025	Fair+	Fair	.....	.....
12. Gibson	1/0.2	.015	Fair+	Fair	.....	Fair—(Pumpkin)
13. Ogden	1/0.1	.02	Good	Fair	Fair	Fair
14. Arksoy 2913	1/0.2	.02	Good—, Fair+	Fair, Fair+	Fair	.....
15. N44-92	3/0.3	.02	Fair+	Fair	Fair	.....
16. N44-774	1/0.1	.02	Good	Fair	Fair	Fair—
17. Roanoke	1/0.1	.015	Good	Fair	Fair	Fair—
18. C.N.S	2/0.2	.02	Fair+	Fair	Fair	.....
19. Acadian	1/0.2	.02	Fair+	Fair—(Mariny)	.....	Poor
20. Mamloxi	1/0.2	.015	Fair+	Fair	.....	Poor(Pumpkin)
21. Blank	1/0.2	.025	Good—, Fair+	Fair, Fair—(Pumpkin)	.....	.....

Five months natural aging at 90°F.
(1) Fair—, Pumpkin.
(2) Fair—, Pumpkin.
(3) Fair—, Mariny.

2. YIELD AND QUALITY OF ALKALI EXTRACTED PROTEIN

Pilot Plant Processing to Obtain Protein

The solvent extracted, well drained flakes (refer to extraction method above) were placed on paper on a heated floor and the solvent allowed to evaporate over night. This unusual method of desolventizing was used in order not to denature any of the protein material by heat. The analyses of the solvent extracted flakes are shown in Table II. The data show fairly uniform pilot plant extraction efficiencies.

The 20 samples of "air desolventized" flakes were extracted with alkali solutions to recover pure protein as follows: 150 grams of solvent-extracted flakes (9% H_2O) were slurried in 2,100 ml. of water at 120°F. Sufficient caustic soda was added to give a final pH of 8.5 and the slurry agitated slowly for 30 minutes, with the temperature kept constant at 120°F. The slurry was then screened through a U. S. 100 screen and the wet meal (containing approximately 90% H_2O) reslurried in 1.2 times its weight of water at 120°F., agitated 15 minutes, and again screened through a U. S. 100 screen. The wet meal from the second extraction was dewatered to 80-91% H_2O by pressing in cheesecloth, then dried in an oven at 250°F. The combined extracts, including that from the pressing operation, were clarified by passing through a Sharples laboratory supercentrifuge, and the protein was precipitated from the clarified extract (at 115°F.) by adjusting the pH to 4.4 with 7½% sulfuric acid, with rapid agitation during the addition of the acid.

The precipitated slurry was allowed to settle overnight at 100-110°F., decanted to 5% solids content, and filtered on a 13-cm. Buechner funnel, with a vacuum of 20-23" Hg. The filter cake (73-75% H_2O) was dried in a forced-draft oven at 250°F. for 24 hours for yield, or granulated through a 4-mesh screen and dried in a forced draft oven at 120°F. for three hours for solubility. The decanted supernatant and the filtrate were discarded.

The protein samples for solubility were ground in a Wiley Laboratory Mill to pass U. S. 40 screen. One gram of the ground sample was slurried in 250 ml. H_2O at 120°F., containing sufficient NaOH to give a final pH (after complete peptization of the protein) of 10.8, and the mixture shaken gently in a 250-ml. mixing cylinder until distinct particles of protein were no longer visible.

Yield of Protein

Table VI shows the pure protein yields from the 20 soybean flakes. The yields varied from 33.1% to 42.7% with 36.7% as the average yield, Roanoke, Ogden, and Arksoy 2913 gave the highest yields of industrial protein.

TABLE VI

Yields of Alkali Extracted Proteins

	A Protein containing 12% H_2O per 100 gm. flks. containing 9% H_2O	B Bone dry pure protein* per 100 gm. flks. containing 9% H_2O
	%	%
A4-107-12	44.0	34.4
Richland	43.4	33.1
Lincoln	45.1	34.8
Chief	47.8	36.3
N44-92	49.6	38.2
Arksoy 2913	51.2	38.9
Gibson	50.4	38.2
C.N.S.	49.5	38.2
N44-774	46.1	34.2
C-463	46.3	35.6
S-100	49.3	38.1
H-5	48.2	37.9
A3K-884	46.5	34.6
A3-176	46.8	34.7
Lincoln (2nd)	45.1	35.7
Earlyana	48.3	37.4
Mamloxi	44.8	34.9
Roanoke	55.6	42.7
Acadian	47.8	37.2
Ogden	50.9	39.3
Earlier results for comparison:		
A4-107-12 (1st)	48.3	34.3
Richland (1st)	47.2	35.3
Lincoln (1st)	49.6	37.5

*Adjusted for pure protein N × 6.25.

The yields reported here are all higher than would be realized in commercial production since the extracted flakes were "air desolventized" at room temperature. In commercial practice final traces of hexane are usually removed from the flakes by steam stripping. Higher desolventizing flake temperature will denature some proteins, resulting in lower yields.

Solubility and Color of Purified Proteins

The color of the purified proteins obtained from all varieties was in each case normal. Satisfactory solution times, as described above, were found on five samples tested. This property of isolated protein depends on protein extraction and processing conditions rather than on bean strain. A uniform solution time of 4½ minutes was obtained on isolated protein from Roanoke, Arksoy 2913, CNS, N44-92, and Chief.

3. AMINO ACID COMPOSITION AND AVAILABILITY OF AMINO ACIDS TO ENZYME IN-VITRO DIGESTION OF SOLVENT EXTRACTED FLAKES FROM 20 VARIETIES OF SOYBEANS

The solvent extracted, "air desolventized," soybean flakes obtained as described above, were split and one fraction set aside for amino acid analysis. The other portion of each sample was "toasted" in the pilot plant, using methods which would simulate mill practices.

The toasting conditions were as follows:

Weight of sample toasted	6 lb.
Moisture of flakes entering toaster	20%
Toasting temperature	**Toasting time**
70°F. to 190°F.	12 minutes
190°F. to 210°F.	3 minutes
210°F. to 220°F.	3 minutes
220°F. to 230°F.	12 minutes
Total	30 minutes
Moisture of flakes after toasting	11%

Toaster open to atmospheric pressure for entire toasting time.

Amino Acid Composition

The amino acid analyses of both the raw and toasted flakes were made by C. M. Lyman and coworkers of the Department of Biochemistry and Nutrition of the Texas Agricultural and Mechanical College. The details of the analytical procedures used are available in publications from the Texas laboratories. The amino acids selected for this work were the 10 considered "essential" for growth (of the rat) and one, present in large amounts, which is considered commercially important as a condiment (Glutamic). Table VII shows the amino acid composition of raw flakes expressed as per cent of the crude protein and Table VIII the same data expressed as per cent of the meal. Inspection of the data in Table VII reveals that the greatest difference between the strains of beans is in their lysine contents. However, the differences do not appear to be significant.

Rat growth tests were conducted with flakes from the strains which showed the largest and smallest amounts of lysine and significant differences were not obtained. It may be concluded that the raw flakes do not differ materially in the percentages of the various amino acids which they contain. Complete amino acid contents of toasted flakes, to ascertain differences which might result from toasting, were determined

TABLE VII

Amino Acid Composition of Solvent Extracted Non-Toasted Flakes Obtained From 20 Varieties of Soybeans, Expressed as Percentage of Crude Protein

Soybean Variety	Protein (N x 6.25)	Arginine	Histidine	Lysine	Tryptophane	Phenylalanine	Threonine	Valine	Leucine	Isoleucine	Methionine	Glutamic Acid
Acadian	48.89	7.75	2.29	6.52	1.51	4.95	3.95	5.44	7.75	5.34	1.43	18.1
Arksoy	50.92	7.56	2.30	6.54	1.45	5.11	3.87	5.30	7.86	5.30	1.39	18.9
A3-176	48.82	7.68	2.25	6.70	1.56	5.02	3.93	5.43	7.90	5.34	1.43	18.6
A3K-884	47.24	8.09	2.26	6.88	1.50	4.87	4.06	5.31	7.85	5.42	1.40	18.5
A4-107-12	47.95	8.01	2.23	6.53	1.48	4.84	3.84	5.28	7.93	5.22	1.34	18.2
Chief	48.23	7.82	2.30	6.59	1.56	5.04	3.96	5.41	7.86	5.20	1.48	18.4
C.N.S.	50.84	7.87	2.36	6.00	1.55	5.11	3.72	5.35	7.59	5.19	1.31	18.4
C-463	47.24	7.54	2.37	6.90	1.61	5.07	3.96	5.40	7.96	5.39	1.45	18.9
Ea lyana	48.81	7.72	2.29	6.74	1.56	5.20	3.98	5.31	7.95	5.31	1.37	18.7
Gibson	48.19	7.49	2.30	6.91	1.54	5.08	3.82	5.31	8.13	5.35	1.41	18.6
H-5	49.42	7.22	2.16	5.97	1.54	5.04	3.58	5.22	7.97	5.24	1.38	17.9
Lincoln	48.86	7.53	2.29	6.67	1.64	5.22	3.91	5.42	8.08	5.32	1.40	18.4
Lincoln No. 3	46.38	7.72	2.33	6.73	1.60	5.17	4.03	5.48	8.45	5.43	1.53	19.2
Mamloxi	49.49	7.96	2.40	7.07	1.44	5.23	3.86	5.34	7.94	5.53	1.50	18.3
N44-92	48.96	7.60	2.49	6.70	1.49	5.17	4.04	5.47	8.17	5.29	1.35	18.5
N44-774	46.38	7.85	2.37	6.73	1.57	5.13	3.99	5.17	8.04	5.28	1.35	17.9
Ogden	47.81	7.49	2.34	6.76	1.42	5.31	3.83	5.31	8.16	5.48	1.28	18.5
Richland	46.38	8.30	2.35	6.47	1.57	4.80	3.84	5.20	7.98	5.18	1.37	18.8
Roanoke	47.40	7.64	2.47	6.48	1.48	5.23	3.88	5.23	8.02	5.15	1.41	19.0
S-100	49.98	7.56	2.52	6.54	1.46	5.12	3.76	5.32	7.98	5.32	1.42	18.6

TABLE VIII

Amino Acid Composition of Solvent-Extracted Non-Toasted Flakes Obtained From 20 Varieties of Soybeans, Expressed as Percentage of the Flakes

Soybean Variety	Protein (N x 6.25)	Arginine	Histidine	Lysine	Tryptophane	Phenylalanine	Threonine	Valine	Leucine	Isoleucine	Methionine	Glutamic Acid
Acadian	48.89	3.79	1.12	3.19	0.74	2.42	1.93	2.66	3.79	2.61	0.70	8.83
Arksoy	50.92	3.85	1.17	3.33	0.74	2.60	1.97	2.71	4.00	2.70	0.71	9.64
A3-176	48.82	3.75	1.10	3.27	0.76	2.45	1.92	2.65	3.86	2.61	0.70	9.08
A3K-884	47.24	3.82	1.07	3.25	0.71	2.30	1.92	2.51	3.71	2.52	0.66	8.75
A4-107-12	47.95	3.84	1.07	3.13	0.71	2.32	1.84	2.53	3.80	2.50	0.64	8.75
Chief	48.23	3.77	1.11	3.18	0.75	2.43	1.91	2.61	3.79	2.51	0.71	8.90
C.N.S	50.84	4.00	1.20	3.05	0.79	2.60	1.89	2.72	3.86	2.64	0.67	9.33
C-463	47.24	3.56	1.12	3.26	0.76	2.40	1.87	2.55	3.76	2.54	0.69	8.91
Earlyana	48.81	3.77	1.12	3.29	0.76	2.54	1.94	2.59	3.88	2.59	0.67	9.15
Gibson	48.19	3.61	1.11	3.33	0.74	2.45	1.84	2.56	3.92	2.58	0.68	8.95
H-5	49.42	3.57	1.07	2.95	0.76	2.49	1.77	2.58	3.94	2.59	0.68	8.86
Lincoln	48.86	3.68	1.12	3.26	0.80	2.55	1.91	2.65	3.95	2.60	0.68	8.99
Lincoln No. 3	46.38	3.58	1.08	3.12	0.74	2.40	1.87	2.54	3.92	2.52	0.71	8.90
Mamloxi	49.49	3.94	1.19	3.50	0.71	2.59	1.91	2.64	3.93	2.74	0.74	9.05
N44-92	48.96	3.72	1.22	3.28	0.73	2.53	1.98	2.68	4.00	2.59	0.66	9.08
N44-774	46.38	3.64	1.10	3.12	0.72	2.38	1.85	2.40	3.73	2.45	0.63	8.29
Ogden	47.81	3.58	1.12	3.23	0.68	2.54	1.83	2.54	3.90	2.62	0.61	8.85
Richland	46.38	3.85	1.09	3.00	0.73	2.23	1.78	2.41	3.70	2.39	0.64	8.72
Roanoke	47.40	3.62	1.17	3.07	0.70	2.48	1.84	2.48	3.80	2.44	0.67	8.99
S-100	49.98	3.78	1.26	3.27	0.73	2.56	1.88	2.66	3.99	2.66	0.71	9.30

for only five strains (Table IX). The quantity of the essential amino acids in raw and toasted flakes from the same strains are similar for all acids except lysine. The small but consistent differences in the amounts of this acid found in the raw and toasted flakes indicates that the toasting destroys some of the lysine. When these results were obtained, it was decided to determine the lysine content of the toasted flakes from all of the strains. The comparative data are in shown in Table X. The data show a small difference between the lysine content of raw and toasted flakes. There is, however, no indication that the lysine in any one strain is more sensitive to toasting than that in the other strains.

All of the data on amino acid composition show that no significant differences were found between the amounts of any of the amino acids in the 20 soybean strains. Thus there is no indication in this work that a strain might be selected and grown for the production of soybean meal with a superior protein.

Enzyme In-Vitro Digestion Tests

For the enzyme digestion tests "toasted" samples of hexane extracted flakes were ground to pass a 60-mesh screen. Digestions were carried out in plugged 250-ml. Erlenmeyer flasks with vigorous agitation and were run at 100°F. One-gram samples were first digested with 7.5 mg. of pepsin (Diffco, N.F., 1:3,000) in 40 ml. of 0.1 N HCl for one hour. The digests were then set to pH 8.5 and 25 ml. of 0.4 M Na_2HPO_4 added, followed by 0.45 gm. pancreatin (Diffco, U.S.P. XII) and 0.15 gm. hog intestinal mucosa (Wilson Laboratories). The digestion at 100° was then continued for 16 hours after which the digests were set to pH 6.8, autoclaved at 15-lb. pressure, and allowed to settle 40 hours before taking samples of supernatants for assay.

All glassware, buffer, and reagents were sterilized before use and aseptic handling was employed to minimize contamination during digestion although the meals and enzymes were not sterilized. Toluene (2 ml.) and chloroform (1 ml.) were added at the beginning of each stage of digestion to inhibit bacterial growth. Amino acids were determined microbiologically, using Leuconostic mesenteroides and titrating the lactic acid produced after three days' incubation. The results of the tests for availability of methionine, lysine, and tryptophane to enzyme digestion in-vitro of "toasted" hexane extracted flakes from the twenty varieties are shown in Table XI. A summary of these results follows:

Amino acid	Total in protein	Amount in the protein available to in-vitro enzyme digestion	
	Average %	*Average %*	*Range %*
Methionine	1.40	0.70	0.57 to 0.84
Lysine	6.62	5.40	4.66 to 6.15
Tryptophane	1.53	1.10	0.97 to 1.19

Methods of this type are new and therefore not fully standardized for accuracy. This particular method sometimes gives results from duplicate samples that vary as much as ± 10%, thus indicating that a range of 20% may be expected. When the results above are considered in this light, it appears certain that there are no significant differences between the varieties in available tryptophane. There seems to be some possibility of significant differences in the availability of methionine and lysine.

TABLE IX

Effect of Toasting on the Amino Acid Content of Soybean Flakes (Values expressed as percentage of the protein)

Amino Acid	A3K-884		C-463		Mamloxi		Richland		Memphis blank		Average	
	Raw	Toasted	Raw	Toasted	Raw	Toasted	Raw	Toasted	Raw	Toasted	Raw	Toasted
Arginine	7.92	7.81	7.56	7.63	7.70	7.86	8.10	8.09	8.03	8.17	7.86	7.91
Glutamic acid	18.2	18.3	18.2	18.2	18.5	18.6	18.2	18.1	18.8	18.4	18.4	18.3
Histidine	2.37	2.43	2.48	2.51	2.50	2.48	2.47	2.37	2.40	2.26	2.44	2.41
Isoleucine	5.42	5.38	5.39	5.42	5.48	5.34	5.18	5.23	5.28	5.21	5.35	5.32
Leucine	7.64	7.77	7.73	7.71	7.80	7.77	7.57	7.47	7.66	7.64	7.68	7.67
Lysine	6.50	6.23	6.90	6.38	7.07	6.49	6.35	6.32	6.56	6.40	6.68	6.36
Methionine	1.38	1.39	1.44	1.48	1.45	1.39	1.38	1.40	1.43	1.40	1.42	1.41
Phenylalanine	4.78	4.80	4.92	5.00	5.30	5.32	4.85	4.75	4.80	4.86	4.93	4.95
Threonine	4.06	4.07	3.96	3.89	3.95	4.17	3.81	3.95	3.95	3.80	3.95	3.98
Tryptophane	1.50	1.50	1.61	1.65	1.45	1.49	1.43	1.48	1.55	1.56	1.51	1.55
Valine	5.23	5.24	5.22	5.25	5.32	5.28	5.16	5.12	5.32	5.38	5.25	5.25

TABLE XI

Availability of Methionine, Lysine, and Tryptophane of Toasted Flakes to Enzyme Digestion in-Vitro

Soybean Variety	Protein in Flakes	Methionine			Lysine			Tryptophane		
		Total in Protein	Available in Protein	Proportion of Total Available	Total in Protein	Available in Protein	Proportion of Total Available	Total in Protein	Available in Protein	Proportion of Total Available
	%	%	%	%	%	%	%	%	%	%
Acadian	48.9	1.43	.61	43	6.52	4.91	75	1.51	1.04	69
Arksoy	50.9	1.39	.65	47	6.54	4.72	72	1.45	1.06	73
A3-176	48.8	1.43	.68	48	6.70	5.33	79	1.56	1.17	75
A3K-884	47.2	1.40	.76	54	6.88	4.66	68	1.50	1.08	72
A4-107-12	48.0	1.34	.63	47	6.53	5.00	77	1.48	1.19	80
Chief	48.2	1.48	.66	45	6.59	5.39	82	1.56	1.12	72
C.N.S	50.8	1.31	.75	57	6.00	4.72	79	1.55	1.06	68
C-463	47.2	1.45	.61	42	6.90	5.94	86	1.61	1.08	67
Earlyana	48.8	1.37	.84	61	6.74	6.15	91	1.56	.7-1.0	51
Gibson	48.2	1.41	.79	56	6.91	4.97	72	1.54	1.12	73
H-5	49.4	1.38	.61	44	5.97	5.67	95	1.54	1.03	67
Lincoln	48.9	1.40	.61	44	6.67	5.73	86	1.64	1.17	71
Lincoln No. 3	46.4	1.53	.78	51	6.73	5.17	77	1.60	1.16	73
Mamloxi	49.5	1.50	.71	47	7.07	5.65	80	1.44	.97	67
N44-92	49.0	1.35	.67	50	6.70	4.90	73	1.49	1.04	70
Ogden	47.8	1.28	.57	45	6.76	5.02	74	1.42	1.13	80
Richland	46.4	1.37	.65	47	6.47	5.18	80	1.57	1.16	74
Roanoke	47.4	1.41	.61	43	6.48	5.49	85	1.48	1.08	73
S-100	50.0	1.42	.72	51	6.54	6.00	92	1.46	1.08	74

Description of 20 Varieties by J. L. Cartter

1. Lincoln was developed cooperatively by the U. S. Regional Soybean Laboratory and the Illinois Experiment Station out of a cross between Mandarin and Manchu.
2. Richland was developed by the Indiana Agricultural Experiment Station as a selection from P. I. 70502.
3. A4-107-12 is a line from a cross between Mukden and Richland developed by the U. S. Regional Soybean Laboratory in cooperation with the Iowa Experiment Station.
4. A3K-884 is an early type being developed by the U. S. Regional Soybean Laboratory and the Iowa Experiment Station out of the cross Richland by Mukden.

TABLE X

Effect of Toasting on the Lysine Content of Soybean Flakes
(Values expressed as percentage of crude protein)

Sample	Raw	Toasted
Acadian	6.52	6.21
Arksoy	6.54	6.43
A3-176	6.70	6.55
A4-107-12	6.53	6.38
Chief	6.59	6.89
C.N.S	6.00	6.07
Earlyana	6.74	6.23
Gibson	6.91	6.42
H-5	6.51	6.11
Lincoln	6.67	6.21
Lincoln No. 3	6.73	6.06
N44-92	6.70	6.18
N44-774	6.73	6.04
Ogden	6.76	6.22
Roanoke	6.48	6.26
S-100	6.54	6.21
Average	6.60	6.28

5. H5 is a selection out of a cross between Mukden and Mandarin developed by the U. S. Regional Soybean Laboratory and the Ohio Experiment Station.
6. C-463 is a selection from a cross between Dunfield and Mansoy developed by the U. S. Regional Soybean Laboratory and the Indiana Station.
7. The variety Chief is a selection of a cross between Illini and Manchu developed by the Illinois Agricultural Experiment Station.
8. Earlyana is a variety produced by the Indiana Experimental Station as a selection from a natural hybrid.
9. A3-176 is a selection from a cross Illini by Dunfield and has been developed cooperatively by the U. S. Regional Soybean Laboratory and the Iowa Experiment Station.
10. Lincoln as above.
11. S-100 is a selection developed by the Mississippi Agricultural Experiment Station from a Rogue or off-type found in a field sample of Illini soybeans.
12. The variety Gibson, developed by the Indiana Station, is a pure line selected out of a cross between the two varieties Midwest and Dunfield.
13. The variety Ogden was selected from a cross between the variety Tokyo and P. I. 54610, a plant introduction number brought into this country directly from the Orient. Ogden was developed by the Tennessee Agricultural Experiment Station.
14. Arksoy 2913 is a strain selected out of the variety Arksoy by the Arkansas Experiment Station.
15. N44-92 is a selection out of a cross between the variety Tokyo and P. I. 54610 developed by the U. S. Regional Soybean Laboratory in cooperation with the North Carolina Agricultural Experiment Station.
16. N44-774 is another selection from the same cross above.
17. The variety Roanoke is a selection from the variety Nanking developed by the U. S. Regional Soybean Laboratory in connection with the North Carolina Station.
18. C.N.S. is a strain selected from the variety Clemson by Mr. Wannamaker of Matthews, South Carolina.
19. The variety Acadian is a strain developed by the Louisiana Experiment Station.
20. Mamloxi is a selection produced by the Mississippi Agricultural Experiment Station out of a cross between Mammoth Yellow and Biloxi.

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