respond to a binary mixture of the expected acids. This can be readily seen by reference to the diagrams which indicate the minimum solidification point a binary mixture of acids may have. As the work of Chibnall *et al* (2) indicates the presence of the C_{26}, C_{28} and C_{30} acids in Chinese insect wax, fraction 2, if separation had been achieved might be expected to be a mixture of C_{26} and C_{28} acids. The minimum solidification point for such a binary mixture would be 82.3° while that actually observed for fraction 2 was 79.75°. This is a clear indication that no worth-while fractionation had been secured.

Despite these data, there is an excellent possibility that by the use of a more efficient fractionating column separation at least into binary mixtures could be accomplished with these higher fatty acids. The fact that use of the ordinary Widmer column led to a certain amount of separation as evidenced by the spread in the melting point of the esters and the solidification points of the acids encourages the view that these acids can be analyzed by ester fractionation combined with the use of solidification-point diagrams. Work by others also tends to indicate that fractionation is possible. Collins (3), in an attempt at preparing pure hexacosanoic acid from Chinese insect wax also tried to fractionate the mixed esters. The use of a fractionating column gave results analogous to those obtained here and molecular distillation gave further separation but did not give pure components. Holde and Bleyburg (5) isolated several of the higher fatty acids from C_{24} to C_{30} by fractional distillation and recrystallization but details of their apparatus are not given.

Summary

The solidification point diagrams of binary mixtures of tetracosanoic to triacontanoic acids have been presented along with a representative synthesis of one of the acids. The possibility of using these diagrams for the analysis of the wax acid mixtures has been discussed, but an attempt to separate the acids of Chinese insect wax by ester fractionation into binary mixtures proved unsuccessful.

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The Viability, Chemical Composition and Internal Microflora of Frost Damaged Soybeans[1]

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Unseasonable early frosts in September, 1942, followed by warm dry weather in the more northerly soybean producing areas of Iowa, Illinois and Minnesota, resulted in much of the crop reaching the market at normal moisture levels but containing varying percentages of immature and frost-damaged beans. As this widespread condition was a new experience since soybeans became a major crop in these areas, questions arose as to the storage behavior, viability, feeding and processing values of such soybeans. In connection with a study of the respiratory behavior of soybeans of varying grade, a limited number of 1942-crop samples were collected. Incidental to the respiration studies, certain analytical data were obtained which appear of interest as an index of the value of immature and frosted soybeans for seed, feeding and industrial purposes. The data include commercial grade, viability, proximate feedingstuffs analyses, acidity and iodine value of the oil, phosphate acidity, amino-acid acidity, nonprotein nitrogen, reducing- and nonreducing sugars. In addition, a qualitative and semiquantitative study was made of the internal aerobic microflora.

Materials and Methods

The experimental material comprised two series of 1942-crop samples, one from Minnesota and the other from Illinois. The Minnesota series consisted of 7 composites, showing increasing increments of immature and frost-damaged kernels, which were prepared from 84 samples obtained from the various growing areas of the state. The Illinois series comprised 7 samples taken at market points from farmers' deliveries.

Size of seed was determined in triplicate by counting and weighing 100 seeds; the weights were calculated to a dry-matter basis. Official grades, including test weight per bushel and damaged kernel content, were supplied by the Minneapolis office of the Grain Products Branch, Food Distribution Administration, U. S. Department of Agriculture. Germination tests were made by the Minnesota Seed Testing Laboratory. Proximate feedingstuffs analyses were carried out by the official methods of the Association of Official Agricultural Chemists (1), with the exception of oil content. This departure involved extracting the ground samples with petroleum ether (Skellysolve F) for 12 hours, regrinding with sand, and re-extracting for an additional 14 hours. Iodine values were calculated from the refractive indices of the oils, determined with a Zeiss refractometer equipped with a water jacketed prism head (n_D^{25} 1.46680 to 1.50210), using the following equation of Majors and Milner (2):

$$\text{Iodine Value} = 8626.877\, n_D^{25} - 12575.226.$$

Oil acidity, phosphate acidity, and amino-acid acidity were determined by the methods outlined by Zeleny and Coleman (3) for cereals. Nonprotein nitrogen

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was measured by the trichloracetic acid method, as described by Becker, Milner and Nagel (4). Reducing and nonreducing sugars were estimated by the alkali-ferricyanide method described for wheat flour by the American Association of Cereal Chemists (5).

The internal microflora were studied by means of a surface-disinfection technique followed by plating the wet beans on sterile nutrient agar. About 250 seeds were dipped in 70% ethanol, followed by immersion in mercuric chloride solution (1 : 1000) for 2 minutes. The beans were then washed with water. In order to minimize contamination with air-borne organisms, the beans were then immersed in 1.5% calcium hypochlorite solution for 5 minutes, from which they were transferred to nutrient agar plates. Readings of the number and types of organisms arising from the seed were made after incubation for 5 days at room temperature.

Results

The seed size, grading and germination data are given in Table I. The Minnesota composites were all of sample grade, whereas only three of the Illinois group were so classified. In both series there is a progressive decrease in viability with increasing damage. Thus, in the Minnesota group, Sample No. 1 containing 10.3% of damaged seeds gave 53% germination whereas Sample No. 7 with 55% damage gave only 5% germination. Seed size was consistently related to percent damage only in the Minnesota group.

TABLE I

Official Grading Results, Seed Size and Germination Data for Soybeans

Sample No.	Grade	Weight of 100 seeds D. M. Basis	Test wt. per bu.	Damaged seeds	Germination
		grams	*lb.*	*pct.*	*pct.*
	MINNESOTA SERIES				
1	Sample Grade Yellow	13.0	58	10.3	53
2	Sample Grade Yellow	14.2	57	13.3	44
3	Sample Grade Yellow	12.3	57	21.0	46
4	Sample Grade Yellow	12.2	57	21.0	27
5	Sample Grade Yellow	11.3	56.5	26.0	29
6	Sample Grade Yellow	10.1	57	41.0	14
7	Sample Grade Yellow	9.7	55	55.0	5
	ILLINOIS SERIES				
10	1 Yellow	12.8	58.5	Trace	84
11	3 Yellow	13.9	59	3.3	74
12	4 Yellow	14.6	57	6.4	74
13	4 Yellow	12.2	58	7.5	57
14	Sample Grade Yellow	13.9	57	10.5	42
15	Sample Grade Yellow	13.5	57.8	12.3	45
16	Sample Grade Yellow	14.0	56	18.0	34

The proximate composition of the samples is recorded in Table II, together with the total digestible nutrients computed therefrom on the assumption that the digestion coefficients are the same for sound, immature, and frost damaged beans. Damage is not reflected in any marked differences in proximate composition, and the T.D.N. values do not indicate that immature and frost-damaged soybeans are of inferior value for feeding purposes. However, crude fiber content shows a fairly regular decrease with increasing damage in the Minnesota series. Although the oil content does not consistently decrease with increasing damage and the total range is narrow (Minnesota samples 1.7%; Illinois samples 2.7%), the most severely damaged samples in each series had the lowest values.

The analyses for miscellaneous chemical factors recorded in Table III show greater differences between the various samples than were indicated by proximate composition. The Minnesota series, which averaged lower in commercial grade than the Illinois samples, was consistently the higher in iodine value of oil and, in addition, showed a trend towards increased values with increasing seed damage. The higher iodine values of the Minnesota grown samples is in line with the observations of Washburn (7) and Cartter and Hopper (8) that soybeans maturing under cool weather conditions or in northerly latitudes produce oils of higher iodine value than those grown under warmer conditions. The observed trend towards higher iodine value with increasing damage in the Minnesota series is doubtless merely a reflection of the fact that the most severely damaged beans originated in areas where conditions of higher moisture and lower temperature prevailed. Immaturity would be expected to result in oil of somewhat lower iodine value than normal since Cartter and Hopper (8) found a slight increase in the iodine value of the oil of soybeans with successive dates of harvest commencing with the full-podded stage.

The titratable acidity of various types of extracts has been applied by many workers as a measure of soundness of cereal grains. In a comprehensive study of the various factors contributing to the acidity of

TABLE II

Proximate Analyses and Total Digestible Nutrients

Sample No.	Damaged seeds	Proximate Composition (Dry Matter Basis)					Total Digestible nutrients [1]
		Protein (N × 6.25)	Oil	Crude fiber	Ash	Nitrogen-free ext. (by difference)	
	Pct.	*Pct.*	*Pct.*	*Pct.*	*Pct.*	*Pct.*	*Pct.*
			MINNESOTA SERIES				
1	10.3	38.6	21.1	6.8	5.4	28.1	97.5
2	13.3	38.6	20.6	6.6	5.5	28.7	96.8
3	21.0	38.2	21.3	6.0	5.5	29.0	97.8
4	21.0	39.8	20.1	5.7	5.4	29.0	96.8
5	26.0	39.5	19.7	6.8	5.5	28.5	95.8
6	41.0	40.5	19.6	5.4	5.5	29.0	96.3
7	55.0	39.7	19.8	4.5	5.7	30.3	96.5
			ILLINOIS SERIES				
10	Trace	33.5	20.8	7.8	5.6	32.3	95.5
11	3.3	41.6	20.5	6.8	5.7	25.4	97.2
12	6.4	39.6	21.5	6.9	5.1	26.9	98.4
13	7.5	40.2	22.2	6.0	5.6	26.0	99.4
14	10.5	38.7	19.5	6.9	7.2	27.7	94.2
15	12.3	42.6	20.8	6.9	5.7	24.0	97.7
16	18.0	41.6	19.8	6.3	5.3	27.0	96.6

[1] Calculated from the following digestion coefficients as given by Morrison (6): Crude protein, 89%; crude fat, 88%; crude fiber 37%; and nitrogen-free extract, 67%.

TABLE III

Miscellaneous Analyses (Dry Matter Basis)

Sample No.	Damaged seeds	Iodine value	Acidity mg. KOH/10 g. Fat Acidity Whole bean	Fat Acidity Oil	Phosphate acidity	Amino acid acidity	Non-protein Nitrogen	Sugars mg./10 g. Total [1]	Reducing [2]	Non-reducing [3]	Reducing sugars as percent of total
	Pct.						mg./10 g.				Pct.
MINNESOTA SERIES											
1	10.3	136.0	3.3	15.6	22.5	39.1	13.9	344	65	279	18.9
2	13.3	136.8	4.0	19.4	22.5	44.5	30.6	362	78	284	21.5
3	21.0	136.8	4.0	18.8	24.8	47.4	25.5	325	76	249	23.4
4	21.0	137.7	3.6	17.9	27.1	51.3	29.7	328	81	247	24.7
5	26.0	137.7	3.5	17.8	30.3	50.8	32.6	346	83	263	24.0
6	41.0	138.3	3.8	19.4	27.3	58.7	35.2	356	81	275	22.8
7	55.0	140.6	5.7	28.8	35.5	71.3	45.9	339	110	229	32.4
ILLINOIS SERIES											
10	Trace	133.1	2.5	12.0	22.2	16.1	15.8	419	86	333	20.5
11	3.3	131.9	6.0	29.3	24.8	29.7	22.9	408	78	330	19.1
12	6.4	129.3	2.2	10.2	25.3	29.8	18.1	436	81	355	18.6
13	7.5	131.3	2.1	9.5	25.9	30.8	23.7	428	78	350	18.2
14	10.5	131.3	3.7	19.0	27.7	32.5	24.2	409	89	320	21.8
15	12.3	131.0	2.6	12.5	29.5	31.4	28.9	401	87	314	21.7
16	18.0	133.1	6.7	33.8	27.4	41.6	31.4	273	93	180	34.1

[1] Sum of analyses for maltose and sucrose. [2] Calculated as maltose. [3] Calculated as sucrose.

such extracts, Zeleny and Coleman (3) have applied techniques which divide the acidic substances into three principal classes: (1) free fatty acids, (2) acid phosphates, and (3) amino acids. These acids are present in small quantities in normal grain and increase as a result of enzymic hydrolysis of fats, phytin and proteins, respectively. In the case of corn and wheat, they found that fat acidity markedly increased with increasing deterioration as measured by commercial grade and by germination. Phosphate acidity increased after deterioration was moderately advanced, while amino acid acidity became greater only in badly damaged grain.

Since studies with wheat have shown that freezing interferes with the normal metabolic processes involved in seed maturation and may act to stimulate certain enzymes (9, 10, 11, 12), a measure of these various types of acidity was of interest in the present study. From Table III it will be observed that the oil and phosphate acidities varied over rather wide ranges, but did not exhibit as consistent increases with damaged seed content as did amino acid acidity. This acidity factor was highly correlated with percent damaged seeds in both the Minnesota and Illinois lots.

The nonprotein nitrogen (trichloracetic acid-soluble nitrogen) values, as would be expected, exhibited similar trends to amino acid acidity.

The sugars present in soybeans are mainly nonreducing. With one exception (Sample No. 16), little variation in total and nonreducing sugars was noted from sample to sample within each series. However, reducing sugars increased fairly consistently from sample to sample in the Minnesota-grown beans.

The amino acid acidity, nonprotein nitrogen and reducing sugar values are in line with published data relative to the effects of frost on the wheat grain (9, 10, 11, 12). When wheat and other seeds mature normally these variables decrease and immaturity alone would result in higher than normal values for these factors; on the other hand, in experiments in which wheat at various stages of early maturity was subjected to varying degrees of frost under controlled conditions, these workers found that frost per se may increase nonprotein nitrogen, and reducing sugar content, as a result of stimulated enzymic activity.

Numerous studies, recently reviewed by Ramstad and Geddes (13), have indicated that microorganisms play an important role in the heating and spoilage of grain. Recent studies by Tervet (14) have shown that certain fungi affect the viability of soybeans stored under unsatisfactory conditions. Frost injury might be expected to render the seed more susceptible to attack by microorganisms and result in an increase in the internal microflora.

TABLE IV

Internal Aerobic Microflora

Sample No.	Damaged seeds	Seeds infected with fungi and bacteria: Alternaria	Fusarium	Miscel. Fungi	Bacteria	Total
	Pct.	Pct.	Pct.	Pct.	Pct.	Pct.
MINNESOTA SERIES						
1	10.3	25	1	1	3	30
2	13.3	18	5	0	0	23
3	21.0	30	8	1	11	50
4	21.0	20	6	0	24	50
5	26.0	27	11	0	13	51
6	41.0	39	3	1	15	58
7	55.0	40	8	2	23	73
ILLINOIS SERIES						
10	Trace	0	0	3	0	3
11	3.3	0	4	2	9	15
12	6.4	6	1	0	0	7
13	7.5	6	0	2	15	23
14	10.5	10	0	2	32	44
15	12.3	1	15	0	0	16
16	18.0	12	25	0	6	43

The data given in Table IV show, in the Minnesota samples, a fairly regular and very pronounced inincrease in the percentage of seeds infected with microorganisms as the percentage of damage became greater. The Illinois series, comprising individual lots from various districts, did not exhibit such regularity. *Alternaria* was the principal fungus in the Minnesota samples, while in the Illinois series, *Alternaria* and *Fusarium* were present in about equal amounts. Bacteria (which were not classified) were found in both series. The technique employed gave information only on aerobic microorganisms. From these findings it would be anticipated that immature and frost damaged soybeans would present a more serious storage problem than sound, high-grade soybeans.

It must be emphasized that these samples were not classified as to variety and they originated in different growing areas so that the differences in the various analytical factors cannot be attributed entirely to immaturity and frost damage. In the Minnesota series, compositing doubtless tended to reduce the influence of varietal and environmental factors; this is indicated by the rather uniform trends in the relations between damaged seed content and several of the variables which were studied. Such uniformity did not appear in the Illinois series, which comprised individual samples. It is clear that the effect of immaturity and frost damage on proximate composition is relatively insignificant. Within the limitations of the utility of proximate analyses as an index of feeding value, there is no relation between damage and the value of soybeans for feeding purposes. Neither do the oil content and iodine values indicate any pronounced inferiority of heavily frost damaged samples for oil production. It is realized, however, that processing value depends upon other factors, such as oil color. While no quantitative measurements were made, an increasing green coloration of the oils was observed with increasing damage.

Although differences in proximate composition were not reflected by damaged seed content, the germination and the internal microfloral content of the seeds were closely related to damage, particularly in the Minnesota series. The higher microfloral content of frost damaged seeds implies that low grade soybeans would be more critical in their storage requirements than sound beans. Whether the meals from such frost damaged beans would also involve a greater storage hazard will depend upon the extent to which the organisms are killed by the processing treatment.

Summary

Seven composite samples of Minnesota grown soybeans and seven individual lots of Illinois soybeans of the 1942 crop containing varying percentages of immature and frost-damaged seeds were subjected to various analyses.

Increased damaged-seed content was accompanied by a marked decrease in viability and by increases in phosphate acidity, amino-acid acidity, nonprotein nitrogen, reducing sugars, and in the internal aerobic microfloral content of the seeds.

Proximate composition of the samples within each series showed little variation. In the Minnesota series, crude fiber content slightly decreased with increasing damage but the total digestible nutrients values were essentially similar for all samples.

The most severely damaged samples in each group were slightly the lowest in test weight per bushel and oil content and were the highest in oil acidity. In the Minnesota series, the iodine value of the oil tended to increase slightly with increasing damage; this is attributed to the fact that the most severe frost damage occurred in growing areas which normally produce oil of high iodine value.

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Book Review

"The Dispensatory of the United States of America," by Horatio C. Wood, Jr., and Arthur Osol; assisted by Heber W. Youngken and Louis Gershenfeld. The 23rd edition, published by J. B. Lippincott Company, Philadelphia, London, Montreal. 1943. XIX + 1881 pp. 7 x 10.5 in. Price $15.00.

A reference book that can go through 23 editions and that has served its field for 110 years has undoubtedly proved its value. It covers the drugs, their formulas, directions for preparation and official test of the U. S. Pharmacopeia XII and National Formulary VII, formulas for clinical laboratory reagents, war emergency replacements, trade names, drugs and preparations of the British Pharmacopeia and much other useful information. The detailed descriptions include chemistry, botany, manufacture, pharmacy, dosage, physiological action, therapeutic uses, and toxic properties.

The book is most valuable to pharmacists. However, many others, as pharmacologists, chemists, biologists, etc., will find it a useful and convenient reference book. Definitions, specifications and descriptions of the various fats, oils, soaps, volatile oils, medicinal oils and ointment bases that are used in the drug trade should not be overlooked by individuals in the fat, oil and soap industry.

M. M. Piskur.