The Flavor Problem of Soybean Oil. VIII. Linolenic Acid[1]

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CIRCUMSTANTIAL evidence has long pointed to linolenic acid as the unstable precursor of what are called "reversion"[3] flavors in soybean oil. Marine, linseed, rapeseed, perilla, and soybean oils, all of which contain appreciable amounts of linolenic acid, are considered to develop these unusual flavors on storage. By contrast, corn, cottonseed, peanut, and olive oils, which are free of linolenic acid, are said to develop "typically" rancid flavors on storage.

The linolenic acid theory of reversion, tenuous and controvertible as it is, has greatly influenced the thinking of research workers. As a result, attempts have been made to eliminate this acid by hydrogenation, by polymerization, and by selective extraction. Studies of a fundamental nature, involving the elimination of non-glyceride constituents by the preparation and purification of soybean methyl esters and by the subsequent resynthesis of the glycerides, have also been conducted by various industrial, academic, and government research agencies. At the University of Pittsburgh, highly purified fat acids from sources other than soybean oil have been combined with glycerol in proportions similar to those occurring in soybean oil (2). In this carefully conducted experiment, as in the numerous unpublished experiments of this and other laboratories, conclusions were drawn with considerable reserve.

The unstable nature of such highly purified synthetic glycerides, the difficulty of eliminating taste principles even from chemically pure compounds, and the inherent subjectivity of organoleptic evaluation methods all require that utmost caution be used in interpreting results.

Evidence given in this paper, bearing on the linolenic acid theory of reversion has been acquired from two sources: a) a study of the flavors after storage of furfural-extracted soybean oil in which the linolenic acid content had been significantly lowered, and b) organoleptic identification studies of stored cottonseed oil into whose glyceride structure linolenic acid had been introduced by the use of an interesterification catalyst.

Results

Furfural-Extracted Oils. In the preceding paper of this series methods of fractionating, degumming, alkali refining, bleaching, and deodorizing were discussed (4). Raffinate fractions ranged in iodine value from 100 to 110, and in linolenic acid content from 2 to 3%. Corresponding extract fractions ranged from 144 to 152 in iodine value, and from 10 to 11% in linolenic acid content. In the first of these experiments it was noted that the raffinates or the low iodine value fractions underwent a different course of flavor deterioration than did unfractionated soybean oil or the extract fractions (4). As experiment followed experiment, this observation on quality of off-flavor was confirmed. The overall conclusions derived from these experiments, as cited in that paper, are that raffinates tend to develop the objectionable soybean flavors to a lesser degree than does the unfractionated oil. Because of the apparent relationship of this conclusion to the linolenic acid theory, it seems wise to present the data leading to this conclusion in detail in the present paper.

Before presenting the evidence, an explanation is in order concerning our organoleptic procedures. We feel a measure of confidence when, in a controlled procedure (3), our tasters return a verdict that flavor of sample A is less intense than that of sample B. We have objective statistical methods to evaluate the validity of their decisions. Such confidence is lacking or greatly diminished however when we approach the problem of qualitative rather than quantitative or intensity description of flavors. Complete freedom must be allowed the taste panel judge in his exercise of intuition or imagination in the number and names of flavors designated. It is because of this liberty that statistical evaluation of qualitative flavor data is necessarily difficult. In Table I, for example, the panel's flavor responses are tabulated for samples of soybean oil and for raffinate and extract fractions after storage for six days at 60°C. Panel members indicate whether the individual flavors are weak, moderate, or strong. This table illustrates type of data which must be collated.

TABLE I

Flavor Responses of Soybean Oil and of Raffinate and Extract Fractions After Storage

Flavor	Sample								
	SBO			Raffinate			Extract		
	W	M	S	W	M	S	W	M	S
Buttery	3			3	1		2	3	
Beany	2	8	2	6	4	2	3	8	2
Rancid[a]	3	2	2	2	8	2	1	4	1
Painty[b]	1			2			1	1	
Grassy[b]	2	2		1			3		
Melony[b]	1	1						2	
Sour	1	1	1	2			2	1	
Sour milk	1						1		1

[a] Rancidity response.
[b] Reversion response.

Arbitrary decisions were inevitable. First, the flavor responses were grouped by distinguishing between rancidity and what would be generally agreed upon as "reversion" flavors, namely, fishy, painty, grassy, and melony responses. Further, the decision was made to give weak, moderate, and strong responses arithmetic weights of 1, 2, and 3, respectively, in calculating the flavor response indices. This weighting was used although our unpublished data show that taste, along with sight and hearing, is an exponential function and the geometric mean therefore would probably have been more valid. For those who may feel that such weighting is unwarranted it may be pointed out that even if only the total number of responses are considered, the conclusions remain unaltered.

The collected response data for five experiments in which stored raffinate samples were compared with

[1] Presented at spring meeting of American Oil Chemists' Society, May 1-3, 1950, in Atlanta, Ga.
[2] One of the laboratories of the Bureau of Agricultural and Industrial Chemistry, Agricultural Research Administration, U. S. Department of Agriculture. Report of a study made under the Research and Marketing Act of 1946.
[3] The term "reversion" is admittedly a misnomer, but can be avoided only with considerable circumlocution.

TABLE II

Collected Flavor Responses for Stored Raffinate and Extract Fractions

Experiment No.	Raffinate						Extract					
	Rancid			Reverted			Rancid			Reverted		
	W	M	S	W	M	S	W	M	S	W	M	S
1	5	2	1	3	0	0	6	3	0	5	2	2
2	4	6	5	3	0	0	9	3	0	9	2	1
3	5	4	5	5	1	0	3	6	0	3	1	2
4	2	8	2	3	0	0	1	4	1	4	3	0
5	2	0	1	0	0	0	12	3	0	7	2	1

corresponding stored extract samples are given in Table II, the data of Table I being listed as Experiment No. 4. The reduced reversion tendency of the raffinates is readily apparent. Flavor responses of the original unfractionated oil are not given, but they are similar to those for the extract fraction. Calculation of the index numbers given in Table III can be illustrated by the raffinate sample in Experiment No. 1 of Table II. The weighted value for rancid responses given in line 1 column 2 of Table III is calculated as follows: $5 \times 1 + 2 \times 2 + 3 \times 1 = 12$. The corresponding value for reversion responses is 3×1 or 3. The ratio of weighted rancid responses to reversion responses for the raffinate sample is 4; the corresponding ratio of responses for the extract sample is 1.

TABLE III

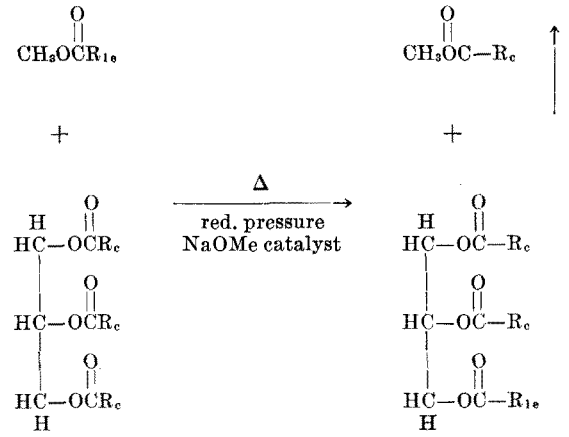
Flavor Response Indices for Raffinate and Extract Fractions After Storage

Experiment No.	Raffinate			Extract		
	Rancid	Reverted	Ratio	Rancid	Reverted	Ratio
1	12	3	4	12	11	1
2	31	3	10	14	14	1
3	28	7	4	15	11	1.3
4	24	3	8	12	10	1.2
5	5	0	∞	18	14	1.3

In the five experiments, as shown in Table III, rancidity responses greatly exceed reversion responses for the raffinate while for the extract fraction, rancid and reversion responses are nearly equal. Unfortunately we know of no statistical method for the evaluation of such data except that whenever any given observation having two choices is repeated five consecutive times, there is 1 in 2^5 or 1 chance in 32 that the same five observations would be made by pure chance. This exceeds the 20 to 1 odds normally considered by statisticians to be significant. It is concluded with statistical justification therefore that raffinates tend less to develop reversion flavors than do the extract fractions or the unfractionated soybean oil.

Since the fractionation of the soybean oil reduces the linolenic acid content from the range of 7-9% to 2-4% in the raffinates, these results bear on the linolenic acid theory of reversion. As has been pointed out (3), and correctly so, other flavor unstable compounds may also have been extracted with the "linolenic acid rich glycerides" so that this evidence is indicative, but not definitive. Although these present observations are in accordance with the linolenic acid theory, they cannot be cited as final proof.

Interesterification Experiments. The second line of evidence is more direct and consists of organoleptic identifications of cottonseed oil into whose glyceride structure linolenic acid has been introduced by means of an interesterification catalyst. The reaction by which this is accomplished may be formulated as follows:



After this reaction the catalyst is "killed" with a calculated amount of diluted hydrochloric acid, the oil is washed with water, and the glycerides are deodorized.

Despite the simplicity of this reaction, considerable difficulty was experienced in obtaining an oil which was initially bland. Results of such an experiment in which all the oils were not initially acceptable are reported in Table IV. In this experiment the methyl

TABLE IV

Number of Identifications on Stored Samples Including Cottonseed Oil Interesterified With 7.5% Methyl Linolenate

Pair	CSO	SBO	Neither	Sig.[1]
CSO	7	1	0	**
CSO-I	7	0	1	
CSO	5	2	0	*
SBO	0	7	0	
SBO	1	8	0	**
CSO-IIe	0	7	2	
CSO	4	5	1	†
CSO-IIe	1	8	1	

[1] † No significant difference.
* Significant difference (5% level).
** Highly significant difference (1% level).

linolenate of high purity was used. It was obtained from the Hormel Institute [4] and prepared by the bromination-debromination procedure. Its spectral constants were slightly higher than those currently used for analytical purposes, and it had an iodine value of 261.4 as compared to the theoretical of 260.4. The crux of the experiment was the organoleptic identification of the cottonseed oil, the soybean oil, and the cottonseed oil interesterified with linolenic acid. The three oils were presented to the panel in pairs. Judges were permitted to identify each sample in one of three ways such as cottonseed oil, soybean oil, or neither.

The first pair of samples consisted of cottonseed (CSO) and cottonseed oil carried through the interesterification process as a control with no linolenic acid added (CSO-I). Both samples were identified as cottonseed oil by seven out of eight tasters. The probability of the seven out of eight tasters agreeing by pure chance is less than 1 in 100. This observation is therefore termed highly significant (**). Had only six out of eight concurred, this would still have been

[4] The mention of this product does not imply that it is endorsed or recommended by the Department of Agriculture over others of a similar nature not mentioned.

significant (*) at the 5% level. It is concluded therefore that the interesterification process does not influence the quality of flavors developed during storage.

The second pair of samples consisted of cottonseed oil and soybean oil (SBO). The identifications were statistically significant at the 5% level.

The third pair constituted a critical experiment since the comparison was between soybean oil and cottonseed oil interesterified with linolenic acid (CSO-ILe). Soybean oil was correctly identified by eight of 10 tasters. The cottonseed oil into whose glyceride structure linolenic acid had been introduced was identified as soybean oil by seven out of 10 tasters. When this cottonseed oil interesterified with linolenic acid (CSO-ILe) was presented in the final pair with cottonseed oil, it was again identified as soybean oil. Unfortunately, and for some unexplained reason, the panel failed to identify stored cottonseed oil in this pair.

At this point in our evaluation work, it was apparent that the samples needed to be initially bland before storage. Considerable time was spent therefore in a study of methods of catalyst preparation and methods of killing the catalyst. Table V shows

TABLE V

Number of Identifications on Stored Samples Including Cottonseed Oil Interesterified With 20% Linseed Methyl Esters

Sample	CSO	SBO	Neither	Sig.[1]
CSO	11	0	1	**
CSO-I	10	0	2	*
CSO-IL	0	9	2	*
SBO	1	8	1	*

[1] For explanation of symbols, see Table IV.

evaluation data upon stored samples which initially were graded as acceptable oils. In this experiment 20% of methyl esters of linseed oil purified by distillation were interesterified into cottonseed oil to give an oil with 9% linolenic acid. The organoleptic identification procedure for this experiment was altered as follows: Three samples were presented at each tasting. One was a stored soybean oil, the second a stored cottonseed oil. Both were identified to the panel members and served them as reference standards. The third sample was the "unknown" whose identifications are listed in Table V. In this as in the previous experiment cottonseed oil, into whose glyceride structure linolenic acid had been introduced (CSO-IL), was identified as soybean oil.

TABLE VI

Number of Identifications on Stored Samples Including Cottonseed Oil Interesterified With 7.5% Methyl Linolenate and 7.5% Methyl Linoleate

Sample	CSO	SBO	Neither	Sig.[1]
SBO	0	6	0	
CSO	5	1	0	**
CSO-ILe	1	5	0	
SBO	1	7	0	
CSO	7	1	0	**
SBO	2	6	0	
SBO	0	7	0	
CSO	7	0	0	**
CSO	7	0	0	
SBO	0	7	0	
CSO	6	1	0	**
CSO-ILo	6	1	0	

[1] For explanation of symbols, see Table IV.

The results reported in Table VI are from experiments in which 7.5% of the purified methyl linolenate was again interesterified with the cottonseed oil. An improved control sample was prepared for this experiment, consisting of cottonseed oil interesterified with methyl linoleate (CSO-ILo). This refinement was suggested to us by the Research Committee of the National Soybean Processors Association with the aim of extending control over the bromination-debromination conditions used in the preparation of the pure methyl linolenate.

Samples were presented to the panel three at a time. In this experiment none of the samples were identified. This reduces the probability of any single taster guessing the correct identification of the three samples to one-eighth. The identification of cottonseed oil interesterified with methyl linolenate (CSO-ILe) as soybean oil was even more positive in this experiment than in the previous two. Cottonseed oil interesterified with methyl linoleate was identified as cottonseed oil rather than soybean oil.

Flavor response data for the first three samples of Table VI are given in Table VII. Rancid and reversion responses are nearly equal for the sample of soybean oil. The pattern of responses for the cottonseed oil interesterified with purified methyl linolenate resembles that for the soybean oil except that this sample was more strongly "reverted." Data similar to these have been obtained in the other two experiments and constitute supporting evidence that linolenic acid is responsible for the fishy, painty, grassy, and melony reversion flavors of soybean oil.

TABLE VII

Flavor Responses After Storage of Cottonseed Oil, Soybean Oil, and Cottonseed Oil Interesterified With 7.5% Methyl Linolenate

Flavor	Sample								
	SBO			CSO			CSO-ILe		
	W	M	S	W	M	S	W	M	S
Beany	3	1		3				1	
Rancid[a]	3	2		2	5	1	3	1	1
Painty[b]	1							5	2
Melony[a]	1		2						
Fishy[b]	1	1					1		1
Metallic	1						1		
Heat reverted	1								
Sour					1				
Stale				2					
Tallowy				1					

[a] Rancidity response.
[b] Reversion response.

Acknowledgment

Fundamental experiments on the flavor problem of soybean oil are frequently simple in concept but difficult to implement. This observation is amply illustrated by the present research. The Engineering and Development Division, which is engaged in the furfural extraction of soybean oil, provided the raffinate and extract fractions used in this study. The Fundamental Oil Investigations Section of the Oil and Protein Division prepared the interesterified oils described, and the Edible Oil Section of the Oil and Protein Division stored the oils and supervised the organoleptic evaluations of the samples. No mention has been made of the unsung heroes of this research, the 19 persons who comprise our present taste panel. Without their continuing interest and support this

study would have been greatly weakened. It is the effective cooperation of these various research groups that has made possible the study of one of the fundamental questions concerning "reversion."

Summary

Circumstantial evidence has long pointed to linolenic acid as the unstable precursor of "reversion" flavors in soybean oil. Direct evidence has now been obtained from two sources: a) A qualitative study of the flavors after storage of soybean oil in which the linolenic acid content has been significantly lowered by furfural extraction, and b) organoleptic identification studies of stored soybean oil, stored cottonseed oil, and a cottonseed oil into whose glyceride structure linolenic acid has been introduced with the use of an interesterification catalyst. It is concluded that linolenic acid is an unstable precursor of "fishy-painty-grassy-melony" flavors in soybean oil.

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[Received August 22, 1950]

The Flavor Problem of Soybean Oil. IX. Organoleptic Identification and Probability Analysis[1]

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IDENTIFICATION of edible oils by their flavor is a difficult problem and is largely an unexplored area in the field of organoleptic evaluation. It is of fundamental importance however to the study of the flavor problem of soybean oil and to the identification of precursors of the undesirable flavors.

In the second paper of this series (4) a procedure designed primarily for measuring intensity or quantitative differences resulting from processing treatments was described. It continues to provide valuable information for that type of problem. However, for identification of oils by virtue of their storage flavors (2), recent research has required the development of additional procedures. This present paper describes such procedures, designed for qualitative flavor study and gives methods of statistically evaluating the results. It is believed that the procedures presented for qualitative study may find application in a variety of organoleptic evaluation problems.

The Identification Problem. One approach to identifying flavor unstable precursors in soybean oil is the introduction of the suspected compound into a relatively flavor stable oil such as cottonseed oil and submitting the simulated soybean oil, after storage, to the taste panel for identification as soybean oil, cottonseed oil, or neither. Patterns for submitting samples to a panel for identification are numerous, and the probability of an individual taster arriving at the correct answer by chance varies greatly with the pattern. A knowledge of this probability permits us to evaluate the identification data objectively. Thus, when the panel response to a given set of samples could occur by chance only once in 20 or more presentations, the result is termed significant (designated as *); if once in 100 or more presentations the result is termed highly significant (designated as **).

Patterns of Presentation and Resultant Probabilities

Samples can be presented in several ways to a taste panel for identification. The simplest method is to present only one sample with alternative answers of A or B, in some cases an answer of neither must be allowed.[3] This test has the same probability for each taster as in the tossing of a coin, i.e., a 50-50 chance.

A second method of presenting samples A and B is the triangle test. Two samples of A and one of B are presented (or two of B and one of A). The tasters are informed that a triangle test is being given and that two of the samples are identical. The judges are asked to select the identical samples. Any person, without tasting, has the probability of 1/3 of selecting the correct pair. Samples could be sorted as A_1A_2-B (the correct answer), A_1B-A_2, or A_2B-A_1.

A third method is to present two samples with the alternative identification of either A or B. In this presentation the possible selections are AA, AB, BA, and BB where the probability of an individual taster giving the correct identification by chance is 1/4.

A fourth method of presentation which results in a lowered probability of correct identification is to increase the number of samples tasted. For example, the taste panel is given two samples each of A and B and then asked to pick out and identify each pair. Types of oils used should be fairly familiar to the tasters. The panel members are told that they are being presented with two pairs of samples so that reporting three of one kind or all of one kind will be avoided. In selecting correctly the two pairs, a random chance selection of samples will give a probability of only 1/6. Only one of the six possible chance selections listed below is correct:

[1] Presented at Spring Meeting of American Oil Chemists' Society, May 1-3, 1950, in Atlanta, Ga.

[2] One of the laboratories of the Bureau of Agricultural and Industrial Chemistry, Agricultural Research Administration, U. S. Department of Agriculture.

[3] From a statistical point of view, to use a 50-50 probability, the alternatives should be either A or B (i.e., cottonseed oil or soybean oil); however, from an experimental point of view, the possibility of foreign flavors must be admitted and the neither response is required. In all experimental work the neither responses were added to the incorrect responses as a conservative measure.