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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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.

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[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.

A_1A_2 and B_1B_2	A_2B_1 and A_1B_2
A_1B_1 and A_2B_2	A_2B_2 and A_1B_1
A_1B_2 and A_2B_1	B_1B_2 and A_1A_2

However if the taste panel members are asked to pick out only the two pairs of samples, with no attempt to identify them as A's or B's, then the probability is 1/3. Both AA - BB and BB - AA are now correct answers.

Similarly, three samples of each type of oil could be given the judges with the requirement to match the triplicate samples and identify the two sets. By increasing the number of identical samples for matching and identification from a single sample to two pairs of different oils, the probability of chance selection drops from 1/2 to 1/6. Going to three samples each of two different oils, the probability drops to 1/20 (8). However, in the case of such a large number of oils (six samples), taste fatigue would not permit the use of such a pattern.

Another variation of the pattern of presentation which we have employed at the Northern Laboratory for the identification of simulated oils is as follows: The judges are presented with three samples, and asked to identify them as either cottonseed or soybean oil. The probability of chance selection for the correct answer in this instance is 1/8 since only two choices are possible for each sample. As there are three samples, the probability is easily calculated ($1/2 \times 1/2 \times 1/2 = 1/8$). In this presentation the identification of the first sample has no effect on the selection of the two remaining samples, and the probabilities are derived by simple multiplication. The possible combinations which can be selected are

AAA	AAB	ABA	ABB
BAA	BAB	BBA	BBB

Note that the above technique using three samples is different from that used in the triangle test, where identification of second and third samples is not independent of the first choice.

From the foregoing considerations it can be seen that probabilities varying from 1/2, 1/3, 1/4, 1/6, 1/8, 1/20 or lower can be selected when evaluating samples of two oils. It all depends on the manner in which the samples are presented. When more than one taster is involved, the same considerations of probability apply, but the larger number of tasters must be taken into account. Expansion of the binomial expression $(p + q)^n$ makes it possible to calculate the compound probabilities for n number of tasters, where

p = probability of success
q = probability of failure
n = number of independent events

$$p + q = 1$$

Considering the possible probability of 1/2 for each of three tasters, it follows that the terms of the expansion of the expression $(p + q)^3$ will give the compound probabilities for three independent events.

$$(p + q)^3 \text{ expands to } p^3 + 3p^2q + 3pq^2 + q^3$$

Substituting 1/2 for p and q:

$$(1/2)^3 + 3\,(1/2)^2\,1/2 + 3\,1/2\,(1/2)^2 + (1/2)^3$$
$$1/8 + 3/8 + 3/8 + 1/8$$

Thus we have a probability of chance selection of 1/8 (first term of the expansion) that all three tasters will make a correct selection. Similarly there are three chances out of eight of making exactly one error (second term); also three chances out of eight of making exactly two errors; and, of course, one chance out of eight of making three errors, that is, of every taster selecting the wrong sample.

Now for 10 tasters the expression for a probability of 1/2 becomes $(1/2 + 1/2)^{10}$, and on expansion this gives 1/1024 + 10/1024 + 45/1024 + 120/1024 + 210/1024 + 252/1024 + 210/1024 + 120/1024 + 45/1024 + 10/1024 + 1/1024. Thus, for a panel of 10 tasters, the probability of all of them selecting the correct sample by chance is 1 in 1024. We also have 10 chances out of 1024 of making exactly 1 wrong selection, and 45 chances out of 1024 of making exactly 2 wrong selections. The probability of making not more than two errors is the sum of these separate probabilities, that is:

$$P = \frac{1 + 10 + 45}{1024} = \frac{56}{1024} = .0547$$

Thus the probability of a panel of 10 members not making more than 2 errors by chance is only slightly higher than that required for a significant result at the 5% level (.05). In other words, to allow a maximum of two wrong answers in a single examination for the selection of sample A over sample B, the panel must have a minimum of 11 members to produce a significant difference between A and B.

Calculation of the number of errors allowed for significance in the case of probabilities of 1/3 is performed by expanding the binomial expression $(1/3 + 2/3)^n$ and the summation of the appropriate number of terms. Similarly, for a lower probability such as 1/8, the chances of guessing correctly are given by the terms of the binomial $(1/8 + 7/8)^n$ which give the compound probabilities expected for n number of tasters. Roessler *et al.* (7) have published a table for significance in triangular tests for 7 to 100 tasters. Gray *et al.* (3) have also plotted similar results for a large number of samplings. Boggs and Hanson (1) have included a formula derived by Snedecor for calculating the number of correct identifications required for significance in the triangle test.

TABLE I
Binomial Expansion for the Probability of 1/2 for 1 to 10 Tasters

Exact No. of Errors	Number of Tasters									
	1	2	3	4	5	6	7	8	9	10
0	1	1	1	1	1	1	1	1	1	1
1	1	2	3	4	5	6	7	8	9	10
2		1	3	6	10	15	21	28	36	45
3			1	4	10	20	35	56	84	120
4				1	5	15	35	70	126	210
5					1	6	21	56	126	252
6						1	7	28	84	210
7							1	8	36	120
8								1	9	45
9									1	10
10										1
2^n	2	4	8	16	32	64	128	256	512	1,024
5% of 2^n	0.10	0.20	0.40	0.80	1.60	3.20	6.40	12.80	25.60	51.20
1% of 2^n	0.02	0.04	0.08	0.16	0.32	0.64	1.28	2.56	5.12	10.20

Table I has been constructed for the probability of 1/2 per taster for 1 to 10 tasters. Values of the binomial coefficient for expansions, where n varies from 1 to 20, are tabulated in most technical handbooks.

TABLE II

Number of Errors Permitted by a Taste Panel to Establish a Significant Difference at the 5% Level

Number of tasters or tastings	Probabilities of				
	1/2	1/3	1/4	1/6	1/8
	Errors allowed cannot exceed				
1	....	....	....	....	....
2	....	....	....	0	0
3	....	0	0	0	1
4	....	0	0	1	1
5	0	1	1	2	2
6	0	1	2	2	3
7	0	2	2	3	4
8	1	2	3	4	4
9	1	3	4	5	5
10	1	3	4	5	6
11	2	4	5	6	7
12	2	4	5	7	7
13	3	5	6	7	8
14	3	5	7	8	9
15	3	6	7	9	10

TABLE III

Number of Errors Permitted by a Taste Panel to Establish a Significant Difference at the 1% Level

Number of tasters or tastings	Probabilities of				
	1/2	1/3	1/4	1/6	1/8
	Errors allowed cannot exceed				
1	....	....	....	....	....
2	....	....	....	....	....
3	....	....	....	0	0
4	....	....	0	0	1
5	....	0	0	1	1
6	....	0	1	2	2
7	0	1	1	2	3
8	0	1	2	3	3
9	0	2	3	4	4
10	0	2	3	4	5
11	1	3	4	5	6
12	1	3	4	6	6
13	2	4	5	6	7
14	2	4	5	7	8
15	2	5	6	8	9

From data in Table I the number of errors allowed for any number of tasters can be calculated by summing up the appropriate figures, as was done in the example for 10 tasters.

Tables II and III show the number of errors that may be allowed for a given number of tasters and a given set of probabilities. These values have been calculated for a significance above the 5% level and the 1% level. Significance at the 1% level cannot be established, at probabilities of 1/2, by panels composed of less than seven members in a single tasting (see Table III). Repeat tastings must be made to establish significance. If two tastings are performed by a panel of six members, one error would be allowed for the 12 tastings. If repeated three times, calculations show that three errors will be allowed. Attendance by panel members varies, and if on one day only six members were present and on the next day eight were present, the total tastings considered will be 14. Note however that the increase in the number of errors allowed is not in direct proportion to the number of tastings. The number of errors allowable in establishing the significance of any experiment can be increased either by increasing the number of tastings and/or by lowering the probabilities of chance selection. These relationships are easily seen in Tables II and III when a comparison is made among the errors allowed for 5, 10, and 15 tasters at the different probabilities.

A recent publication (5) gives the binomial probability distribution for probabilities of 0.01 to 0.99 and for numbers up to and including 49 tasters. These data can be used to extend Tables II and III for taste panels having a greater number of tasters or requiring probabilities other than those presented.

Example of Application

Actual data obtained with a taste panel trained in the evaluation of edible oils will illustrate the application of the probability tables. In the identification of simulated soybean oil, the taste panel judges were selected on the basis of their ability to identify aged cottonseed oil 80% of the time. Some excellent tasters, as judged from scoring data, failed to be included in this panel because of high errors in previous identification tests on both soybean and cottonseed oil.

TABLE IV

Taste Panel Identifications of Cottonseed, Soybean, and Simulated Soybean Oils

Taster No.	Warm-up Samples		Test Samples			
	I C S C[1]	II C S S	III S C (C–Le)[2]	IV S C S	V C (C–Lo)[3]–S	VI C C S
	Panel Identification					
1	C S C	C S S	S C S	S C S	C C S	C C S
2	C S C	C S S	....	S C S	C C S	C C S
3	C S C	C (N)(N)	S C S	(C) C (C)	....	....
4	C S C	C S S	S C (C)	S C S	C C S	C C S
5	C S C	C (C) S	S C S	....	C C S	C C S
6	C S C	C S S	....	S C S	C C S	C C S
7	C S C	C (C) S	....	S C (C)	....	C C S
8	....	(S) S S	S (S) S	S (S) S	(S)(S) S	....
9	C S C	C S S	....	....	....	....
10	C S C	C S S	S C S	S C S	C C S	C C S
Probabilities	.002	.0005	.003	.0012	2×10^{-5}	5×10^{-7}

[1] First two samples in Test I only. Identified to the panel as cottonseed oil and soybean oil, in all other tests all three samples presented as unknowns.
[2] C–Le cottonseed oil interesterified with methyl linolenate.
[3] C–Lo cottonseed oil interesterified with methyl linoleate.

In the evaluations shown in Table IV an answer of "neither" was regarded as an error and served to lower the significance of the results as noted in footnote 3. Columns I and II show the results of two indoctrination tests (warm-up samples) and columns III to VI show the identifications of simulated oils and their controls. Results given in column I are from the presentation of one unknown with known samples of soybean oil and cottonseed oil. This manner of presentation gives the probability of chance selection of 1/2 while in the remaining presentations, columns II to VI, all three samples were presented as unknowns, and the probability of chance selection was 1/8. The errors in identification are the encircled responses shown scattered throughout the table. The probability of this number of judges being in error by chance is shown in the last line of the table. In calculating the probability of the error, *it is the number of tasters making errors in identification of the samples presented for a unit tasting that must be considered.* In order to use the probability of 1/8 the results of each taster must be considered as a success or a failure. Thus a taster making one error in judgment is no better than a taster giving two wrong responses. Incidentally, the person making only one error may be a better taster, but to consider individual errors the probability of the experiment must be lowered from 1/8 to 1/2.

The results presented in column IV, Table IV, show that a total of 3 out of 8 tasters erred in the identification of the three oils. From Table III it is seen that these data are highly significant (1% level), and the actual probabilities that this result could be obtained by pure chance are computed as 1 in 813. If each oil in this presentation (column IV, Table IV) were considered as being presented individually (probability 1/2), the first and second samples would exhibit significant results only (5% level), and the third sample would be nonsignificant. These results show that when a small number of tasters are employed, it is more difficult to establish significant differences in samples with a chance probability of only 1/2.

The binomial expansion is of limited value in applied statistics for two reasons (6). It is not a continuous curve, and when n is large, the size of the numbers involved in the calculations is too great to be handled. However, for small samples and for probabilities of success appreciably smaller than 1/2, the normal frequency curve fails to give the exact probabilities in which we are interested. We must rely therefore upon the binomial expansion.

Summary

In the evaluation or identification of an edible oil it is shown how the probabilities of chance selection for a single taster are dependent upon the manner in which the samples are presented. The probabilities of 1/2, 1/3, 1/4, 1/6, and 1/8 are all possible, depending upon the method of sample presentation, i.e., as pairs, multiple pairs, odd sample tests, or other combinations.

The expansion of the binomial expression for the probabilities of success and failure for a single taster make it possible to calculate the compound probabilities for taste panels up to 20 members. By knowing the compound probabilities, significance at any level can be easily calculated. Tables showing the number of errors permissible have been calculated for the significant levels of 1% and 5% for panels of 1 to 15 members.

Application and use of the tables, based on data obtained in the identification of simulated soybean oil by a taste panel of 10 members, are presented and the data discussed.

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A Comparison of Several Methods for the Separation of Unsaponifiable Material From Carnauba and Sorghum Grain Waxes[1,2]

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A WHITE crystalline solid isolated (1) from the lipid fraction of sorghum grain subsequently was shown to be a wax which had properties similar to those of carnauba wax (2). In the present investigation several different methods of separating the components of waxes were applied to carnauba and sorghum grain waxes. The results obtained were correlated with the constants for carnauba wax and four different varieties of sorghum grain wax.

Experimental

Purification of solvents. The Skellysolve B, isopropyl ether, benzene, ethyl ether, and acetone used in these experiments were dried over $CaCl_2$ and distilled in an all-glass apparatus.

Extraction and purification of sorghum grain wax. The wax was extracted from either the whole grain or bran. The whole grain was extracted in 5-lb. batches by refluxing on a steam cone for 1 hr. with 2 l. of Skellysolve B. The hot extract was filtered, cooled to 0°C., and the resulting precipitate removed with the aid of a Buchner funnel. Yields of about 0.25% wax were obtained. The sorghum bran was extracted in a large Soxhlet extractor with Skellysolve B for 20 hrs. Bran from the Cody variety yielded 6% of crude wax based on the weight of the bran.

The crude wax was purified by recrystallization from a mixed solvent. A solution of 50 gm. of crude wax in 2 l. of Skellysolve B was filtered through a water-jacketed filter at 70°C., one liter of solvent removed by distillation, 2 l. of acetone added, and the solution placed in a refrigerator at 0°C. for 24 hrs. The purified wax was recovered by filtration. The yield was in the range of 80 to 85% for the varieties tested.

Preparation of calcium stearate. Stearic acid was synthesized from U.S.P. castor oil by the method of Schuette and Roth (3) and converted to the calcium salt.

Determination of wax constants. Acid values were determined by titration of a hot solution of 0.5 gm. of wax in 50 ml. of ethanol and 10 ml. of toluene with 0.05 N KOH in alcohol. The titrations were carried out in an Erlenmeyer flask equipped with a side arm condenser and stirred with a magnetic paddle. Acetyl values were determined by the alkalimetric method of Roberts and Schuette (4), modified to employ a smaller sample. This method was checked by the iodimetric method of Elek and Harte (5). When sufficient sample was available, quintuplicate deter-

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