added by drops to the stirred mixture. The temperature was held at 175°–185°F. for 6 hrs., then dilute HCl was added until slightly acidic. Sodium chloride and excess HCl were removed by washing with water. The remaining water was removed under vacuum. The product was a yellow waxy material which would not cure to a hard resin when admixed with ethylene diamine, diethylene triamine, triethylene tetraamine, or phthalic anhydride at baking temperatures.

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A Determination of the Effects of Several Variables on the Performance Characteristics of Shortening, Using Statistical Experimental Designs

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To obtain shortening of higher water absorption, one should increase the level of monoglyceride used in the shortening. Icing volume is adversely affected by higher levels of monoglycerides. It is immaterial whether the monoglyceride has 40–44% or 56–58% *alpha* mono content, and the I.V. may range from 56 to 90 without producing significant effects on water absorption, icing volume, or cake results. Tempering and the choice of vegetable fat or rearranged meat fat stocks are of importance.

I. Statistical Experimental Design and Its Applications

THIS PAPER will present a simple outline of procedure used in a shortening study where experimentation was on a statistical basis to achieve maximum results in a minimum of experimental time.

While the use of statistical methods in production and quality control is widely accepted, we find that some of the statistical techniques available are not well known to the research and development chemist. It is hoped that this presentation will stimulate further investigation.

Much chemical experimentation has to deal with systems made up of a number of variables, in which the chemist has to determine the proper conditions for each variable necessary to achieve optimum results. One common way to do this would be to take one particular ingredient (or factor), vary it to find the level which works best, then hold that optimum condition fixed and vary some other factor: find its optimum, and go on to another variable. There are some cases however for which this is not the most efficient way to proceed.

Statistically it is possible to show that we can get more accurate results, in a shorter period of man-hours, by applying some of the theories of statistical experimental design. This is an orderly, planned experiment in which we have decided how to vary the factors we wish to study in relation to one another. Under this approach all variables are changed simultaneously instead of only one factor at a time.

The advantages of changing all variables simultaneously according to a definite plan as opposed to the "one-varying, others-fixed" approach can be summed up as follows. We are able to determine differences in end-results that are smaller than would be apparent if only one item were varied at a time. This approach brings to light the changes in end-results caused by the interaction of variables, a factor hidden if only one at a time were moving. The use of this approach enables one to make an accurate estimate of the inbred experimental error in our procedure, a factor difficult to determine in common procedures without running a number of separate repetitive experiments.

We can chart a typical chemical problem of four variables (Table I). By assuming that our final answer will be made up of some combination of the varying levels of use of this material, we can quickly determine the number of total runs required to conduct a complete factorial series. With one variable having four possible levels of use, two other variables each with three levels of use, and a fourth ingredient having only two levels of use, we would have 72 distinct experimental runs to perform.

TABLE I

A Factorial Experiment

Variables	Code	Levels for use	Total number of levels
Alpha mono in monoglyceride	A	40 48 56	3
Iodine value	B	50 70 90	3
Alpha mono in shortening	C	1.75 2.00 2.25 2.50	4
Tempering	D	Absent Present	2

It is satisfactory, in a majority of cases, to run just two levels of use for each of the four factors, the high limit and the low limit, for each of the four variables and, by graphical and mathematical means, to consolidate the data thus gained to fill in what we might not have derived directly from experimentation. Two-level experiments are simple to analyze without the need for high-speed computers. Furthermore they lend themselves to fractionation. This means that if there are five factors in an experiment and it is wished to keep the number of runs to a minimum, the appropriate 16 runs will supply all pertinent information whereas the complete two-level design would require $2^5 = 32$ runs.

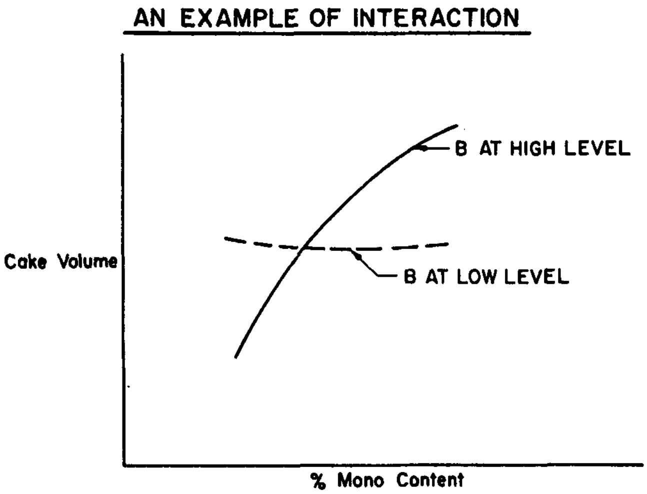

FIG. 1.

Figure 1 shows the effect of varying the monoglyceride content of a cake shortening in terms of volume of the cake. Under the "one-factor-at-a-time" system mono content would be varied, but other factors would be held constant. If the low level of Factor B were chosen, we would erroneously conclude that varying the mono content did not affect cake volume. If, on the other hand, the high level of B were selected, we would see a distinct relationship between mono content and cake volume. Statistical design shows that mono content and Factor B work together in affecting cake volume. This phenomenon is called an interaction.

TABLE II

The 2-Level Factorial

Code	Factors	Conditions	
		Low	High
A	*Alpha* mono in monoglyceride	40.00	56.00
B	Iodine value of monoglyceride	56.00	90.00
C	*Alpha* mono in final shortening	1.75	2.50
D	Tempering	Absent	Present

Total runs $= 2 \times 2 \times 2 \times 2 = 2^4 = 16$.

Let's now follow a typical case through to conclusion. Referring to Table II, we see that we are dealing with four variables, each having a high and a low level. The number of runs required will then be $2 \times 2 \times 2 \times 2$ or 16. This particular experiment was set up to determine the effect of varying shortening ingredients on icing volume but could be extended to other forms of chemical experimentation.

The first step is to list the manner in which the variables are to be manipulated (Table III). One way to indicate the levels of each variable in each experimental run is to set up a symbolic code in small letters. Each letter in lower case corresponds to the variable originally indicated in capital letters. But, instead of simply indicating the variable, it is now used to indicate the level of use of that variable in the experimental run. In other words, where the lower case "a" appears, it indicates that the high level of the variable "A" is used. The complete absence of a lower-case letter in our symbolic code indicates that the run was made with the low level of that ingredient. The use of a code makes it easy to tell that all of the possible combinations of variables needed to complete the statistical design have been included.

TABLE III

		A	B	C	D
Combination code	Emulsifier	*Alpha* mono content (%)	I.V.	Level of use % *alpha* mono	Tempering
(1)	1	40.5	58	1.75	Absent
a	2	55.7	56	1.75	Absent
b	3	40.5	90	1.75	Absent
ab	4	55.7	90	1.75	Absent
c	1	40.5	58	2.5	Absent
ac	2	55.7	56	2.5	Absent
bc	3	40.5	90	2.5	Absent
abc	4	55.7	90	2.5	Absent
d	1	40.5	58	1.75	Present
ad	2	55.7	56	1.75	Present
bd	3	40.5	90	1.75	Present
abd	4	55.7	90	1.75	Present
cd	1	40.5	58	2.5	Present
acd	2	55.7	56	2.5	Present
bcd	3	40.5	90	2.5	Present
abcd	4	55.7	90	2.5	Present

We are now ready to perform the actual experimental work. The coded results of the experimental work are shown under the column labelled Percentage in Table IV. This column gives the water absorption obtained for each of the 16 experimental runs. In order to make calculations easier, 500% has been subtracted from each of the 16 results. Such coding by subtracting a number from all data will not affect our final answers.

TABLE IV

Yates Analysis

Treatment combination	Percentage	(1)	(2)	(3)	(4)	Effect
(1)	—27.0	19.0	998.5	2050.5	3815	238.4
a	46.0	979.5	1052.0	1764.5	—119	— 14.9 A
b	487.5	108.5	634.2	34.5	2614	326.8 B
ab	492.0	943.5	1130.0	—153.5	—481	— 60.1 AB
c	53.5	228.5	77.5	1796.5	549	68.6 C
ac	55.0	406.0	— 43.0	817.5	251	31.4 AC
bc	494.0	245.0	—262.5	—114.5	337	42.1 BC
abc	449.5	885.0	109.0	—366.5	303	37.9 ABC
d	99.0	73.0	960.5	53.5	—286	— 35.8 D
ad	129.5	4.5	835.0	495.5	—188	— 23.5 AD
bd	349.5	1.5	177.5	—120.5	—979	—122.4 BD
abd	56.5	— 44.5	640.0	371.5	—252	— 31.5 ABD
cd	84.5	30.5	— 68.5	—125.5	442	55.3 CD
acd	160.5	—293.0	— 46.0	462.5	492	61.5 ACD
bcd	426.0	76.0	—323.5	22.5	588	73.5 BCD
abcd	459.0	33.0	— 43.0	280.5	258	32.3 ABCD

The mathematical treatment of the data is simple and methodical. A discussion of the computation is presented in Davies (8). The values in the effect column represent the estimated change in water absorption corresponding to each capital letter in the far right-hand column.

The real meaning of the numbers obtained for each of the effects or mean squares is masked because of the experimental error of the system. Relative comparisons of the numbers must be made to establish whether, say, the —14.9 obtained as the effect of A is due to changing A (% α mono content) from low to high level (40 to 56).

The conventional way of doing this is by analysis of variance (Table VI). The mean squares for each main effect and interaction of two factors are tabulated. All mean squares corresponding to terms of interactions with more than two factors are averaged to form the error term. In this example there are five mean squares in the error term.

TABLE V

Ranking of Effects

Ranking probability	Ranking of effects
3.4	14.9
10.0	23.5
16.7	31.4
23.4	31.5
30.0	32.3
36.7	35.8
43.4	37.9
50.0	42.1
56.7	55.3
63.4	60.1
70.0	61.5
76.7	68.6
83.4	73.5
90.0	122.4
96.7	326.8

The magnitude of the mean squares in Table VI are all compared to the error term. Statistical theory tells us that these mean squares can be expected to vary just by chance. By use of the F test we can test to see if the variation is due to chance. In our example the F test indicates that a value as large as 1,070,400 could be obtained by chance. Only the Factor B mean square is larger. This indicates that Factor B is the only detectable variable related to water absorption. In Table IV the estimate of the B effect was 326.8. Statistics show that the magnitude of the other effects could be attributed just to experimental variations. The interested reader is again referred to Davies for a detailed discussion of these techniques.

TABLE VI

Analysis of Variance

Source of variation	Degree of freedom	Mean squares
A	1	14,161
B	1	6,832,996**
C	1	301,401
D	1	81,796
AB	1	231,361
AC	1	63,001
BC	1	113,569
AD	1	35,344
BD	1	958,441
CD	1	195,364
Error	5	161,937

Critical F test at 95% = 6.61 × error = 1,070,400.

Another statistical procedure (7) has been developed for making decisions of this nature. To apply this graphical method we first rank the effects from Table IV in order of ascending absolute magnitude. The ranking is shown in Table V. A ranking probability, expressed as percentage, is attached to each of the rankings. This is obtained from the formula $\frac{i - 1/2}{n}$, where n is the total number of rankings (15 in our case) and i is the particular ranking. The value of i can vary from 1 up to n.

These pairings can then be plotted on half normal-probability paper (Figure 2). If the magnitude of the effects just result from experimental variation, these points should all cluster around a straight line. When significant effects are present, they will represent the larger rankings and fall well off the line estimated for the other rankings.

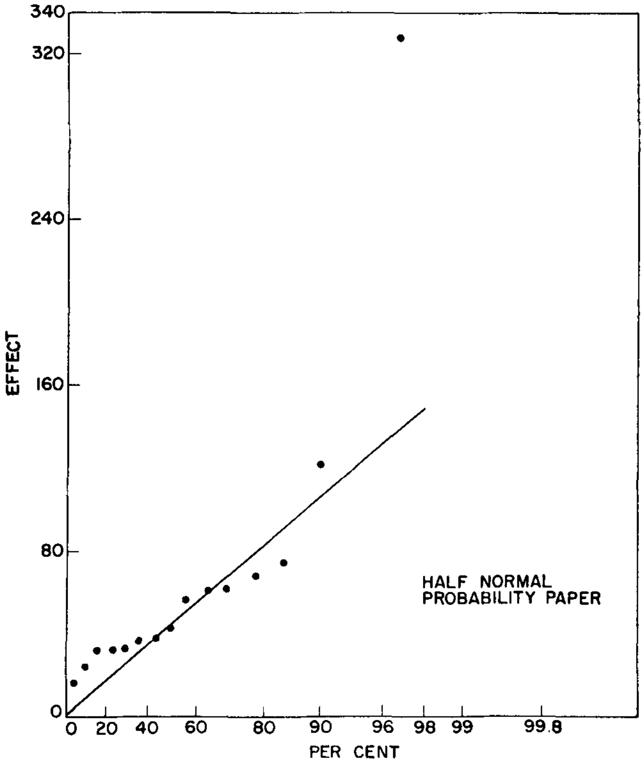

FIG. 2. Half-normal graphical analysis: water absorption results with rearranged meat fat coded data.

In Figure 2 only one point falls well off the straight line which was fitted to the other points. This effect is important. Reviewing Table IV, we see it is Factor B. Additional information on these graphical methods may be obtained from an article by Daniel (7).

The two techniques of analysis may be used jointly so that erroneous decisions are minimized. The final conclusion is that, of the four factors which were varied, only B created a significant effect.

We have attempted to convey some of the concepts behind experimental design, particularly two-level factorials. Perhaps this introduction to experimental design will allow a fuller understanding of the discussion on the use of factorial designs in the experiment described in Part II of this paper.

II. The Factorial Experiment

THE EFFECTS of individual variables on the performance characteristics of shortening have been considered by several authors. Swanson (1) discussed this under three categories: handling, quality, and stability, as applied to shortenings for dry mixes. He listed water-emulsifying ability as an important test of the quality of a shortening. He also considered the effect of fatty acid, type of shortening, amount of shortening, level of monoglyceride emulsifier, type of emulsifier, *i.e.*, hard or soft, and ratio of monoglycerides to diglycerides. These factors were all considered individually, and there was evidently no attempt to determine the interactions, if any. Bailey and McKinney (2) developed a "rational" method for evaluating the creaming qualities of shortening.

Stingley and Wheeler (3) discussed the meaning of commonly-quoted values as related to shortening performance, stating that some of the various chemical and physical constants of a shortening can be translated by an experienced operator into approximate terms of shortening performance, but, in order actually to prove what a shortening will do, performance tests are required. They described briefly the following tests: pound cake, wet-creaming volume, icing volume, layer cake, water absorption, dry-creaming volume, and prepared mix.

Kuhrt and Welch (4) have shown that cake-volume measurements can be sufficiently precise to use in the evaluation of monoglycerides. They also report that different types of monoglycerides have been shown to possess marked differences in their activity in cake baking, observing improved activity when monoesters made from fats containing unsaturated acids were used. They conclude that in a water-absorption test the unsaturated fatty acid monoglycerides are considerably more active than the monoesters of saturated fatty acids.

An eminently valid but apparently disregarded conclusion was reported by Harris, Epstein, and Cahn (5) in 1941, wherein it was stated unequivocally, "It is commonly and erroneously accepted by many in the industry that the amount of water which can be emulsified in a shortening is a measure of the efficiency of the shortening in the production of cakes with increased proportions of sugar and moisture." In spite of this early appraisal many large consumers of high-absorption shortening include a water-absorption requirement as one of their specifications.

A second test usually applied is icing volume, which is of vital concern to the baker who wishes to stock a minimum number of shortenings.

The effects of four factors that may be regulated in the production of shortening were examined: the monoglyceride content of the emulsifier, the hardness or plasticity of the emulsifier as indicated by its I.V., the level of use of the emulsifier, and the effect of tempering or not tempering the resultant shortening.

Several criteria of commercial importance were considered in adjudging the effects of varying these factors on the performance of the resultant shortening. The performance tests were: icing volume, cake baking, water absorption. The icing-volume and water-absorption tests are standard methods.

In the cake-baking tests standard white, yellow, and devil's food cakes were baked, and the volume in cc. of two layers of a scaled weight of batter in each as well as the over-all cake scores are reported. The cake formulas used are given in Table VII and the scoring method in Table VIII.

In spite of doubts as to the significance of water-absorption results, as corroborated by Stingley and Wheeler, and Harris *et al.*, the effects of varying the several factors on this property were determined.

METHOD

Icing-Volume Determination

Formula

Powdered 10X sugar (sifted)	681.0 g.
Shortening	170.3 g.
Distilled water	128.0 cc.

TABLE VII

Cake Formulas

Ingredients (g.)	130% White cake	Yellow cake	Devil's food cake
Cake flour	181.6	181.6	181.6
Sugar	236.1	236.1	236.1
N.F.D.M.S.	18.2	22.7	31.7
Salt	4.53	3.4	5.66
Baking powder	9.9	9.06	9.06
Baking soda	......	......	3.4
Cream of tartar	2.55	......	......
Breakfast cocoa	......	......	36.3
Emulsifier shortening	90.8	77.7	80.6
Water	99.8	100.0	127.0
Water	27.2	36.3	45.4
Water	......	18.2	50.0
Whole eggs	13.2	45.4	99.8
Egg yolks	......	72.6	......
Egg whites	122.5		......
Totals	806.78	803.06	906.62

Scale 350 g. into two 8-in. pans.

TABLE VIII

Cake-Baking Test Report

External			Internal	
Batter mixed °F.	Volume	20	Grain	25
Batter viscosity	Color of crust	5	Color of crumb	10
Batter Specific gravity	Symmetry of form	5	Aroma	5
Batter appearance	Character of crust	5	Taste	10
Cake volume, cc.	Total	35	Texture	15
Cake weight, g.			Total	35
Total cake score				100

Procedure. Place sugar, shortening, and half of the water in a 3-qt. bowl, using a paddle type of beater; mix ingredients at speed #1 (144 r.p.m.) until a thick paste is formed.

Change mixer to speed #2 (258 r.p.m.); and after 2 min. add remaining water slowly over a period of 3 min.

Scrape down bowl. Continue creaming for a total of 24 min., taking a weight sample at 10 min., 15 min., 20 min., and 24 min. in a calibrated density cup.

Calibration of Density Cup. A stainless steel cup with a volume of 234 cc. is used. The volume of the cup is not critical, but a volume of at least 200 cc. is recommended.

Calculation

$$\frac{\text{Volume of cup}}{\text{Weight of sample}} \times 100 = 100 \text{ volume of icing in cc. per 100 g.}$$

METHOD

Water-Absorption Determination

Weigh 3-qt. mixer bowl and paddle beater. Add 200 g. of shortening. Set mixer on speed #2 (250 r.p.m.), and agitate for 30 sec. Begin adding water at the rate of 20 cc. per minute (continuously from a 2-liter flask suspended on a ring stand above the bowl and added to the bowl by siphoning) until the emulsion breaks. Record time when completed, and weigh bowl, beater, shortening, and water.

Example

(Start) Weight of bowl and beater............ 898 g.
shortening............ 200 g.
1098 g. (Total)

(Finish) Weight of bowl, beater, shortening, and water..............................2608 g.
Weight of bowl, beater, and shortening....................................1098 g.

Weight of H_2O...............................1510 g.

For 200 g. of shortening,

$$\frac{1510}{2} = 755\%$$

Experimental

Some of the factors amenable to control in the production of shortening may be designated as follows:

A—mono content of the monoglyceride *(alpha)*
B—iodine value (I.V.) of monoglyceride
C—level of addition in shortening
D—tempering

There had been indications that a blend of the monoglycerides at the two levels used in B was superior to either one or a single monoglyceride derivative of I.V., equivalent to that of the blend, so an additional experiment was conducted in which the high and low level of B were blended to give, in effect, a third level of B.

To obtain two levels of monoglyceride content and I.V., four monoglycerides were prepared as shown in Table IX.

TABLE IX

	Alpha mono content, %	I.V. of monoglyceride
Monoglyceride 1	40.5	58
Monoglyceride 2	55.7	56
Monoglyceride 3	43.4	90
Monoglyceride 4	57.2	90

For the second experiment in which a third level of Factor B was examined, 1 and 3 and 2 and 4 were blended to give an I.V. of approximately 75.

For Factor D eight samples were used without tempering, and eight of the samples were "tempered" for 24 hrs. in an 86°F. cabinet and allowed to equilibrate at room temperature, 75°F., before using.

The levels at which each factor was examined in all combinations were:

	Low level	High level
A	40–44% *alpha* mono content	55–57% *alpha* mono content
B	56–58 I.V.	90 I.V.
C	1.75% *alpha* mono added	2.5% *alpha* mono added
D	not tempered (−)	tempered (+)

These levels were chosen on the basis of the type of products generally used in high-absorption baker's shortenings. This type of shortening represents, at best, a compromise for all general bakery use except frying. If cake volume and quality are the only criteria of performance, a lower range of iodine values and higher use levels have been shown to be beneficial by such authors as Swanson, Kuhrt, and others. Products of much lower I.V. or conversely much higher titer than the products represented have been shown to have very deleterious effects on water absorption and icing volume.

In this experiment a standard hydrogenated vegetable oil shortening (Kopald[1]) was used as the base stock. Treatment combinations are listed in Table X. Where the number (1) appears, all factors are at the lower level. Where the small letter a, b, c, d appears, it means the factor is at the higher level.

Sixteen additional combinations were examined, using a blend of the high mono, low and high I.V. monoglycerides, and the low mono, low and high I.V. monoglycerides. Eight of these combinations were tested with hydrogenated vegetable oil shortening (Kopald), and eight with a new Factor "E," a rearranged meat fat shortening (Purity).[2]

The blend of the low and high I.V. monoglycerides make a common I.V. of about 75. Therefore the Factor "B" was eliminated in the coding of the following treatment combinations:

TABLE X (1)

Combination code	Emulsifier	Emulsifier mono-content, %	I.V.	Level of use % *a* mono added	Tempering
(1)	1	40.5	58	1.75	Absent
a	2	55.7	56	1.75	Absent
b	3	40.5	90	1.75	Absent
ab	4	55.7	90	1.75	Absent
c	1	40.5	58	2.5	Absent
ac	2	55.7	56	2.5	Absent
bc	3	40.5	90	2.5	Absent
abc	4	55.7	90	2.5	Absent
d	1	40.5	58	1.75	Present
ad	2	44.7	56	1.75	Present
bd	3	40.5	90	1.75	Present
abd	4	55.7	90	1.75	Present
cd	1	40.5	58	2.5	Present
acd	2	55.7	56	2.5	Present
bcd	3	40.5	58	2.5	Present
abcd	4	55.7	90	2.5	Present

TABLE X (2)

Combination code	Emulsifier	Emulsifier mono-content, %	Level of use % *a* mono added	Tempering	Shortening base stock
(1)	1 + 3	40.5	1.75	Absent	Vegetable
a	2 + 4	55.7	1.75	Absent	Vegetable
c	1 + 3	40.5	2.5	Absent	Vegetable
ac	2 + 4	55.7	2.5	Absent	Vegetable
d	1 + 3	40.5	1.75	Present	Vegetable
ad	2 + 4	55.7	1.75	Present	Vegetable
cd	1 + 3	40.5	2.5	Present	Vegetable
acd	2 + 4	55.7	2.5	Present	Vegetable
e	1 + 3	40.5	1.75	Absent	Animal
ae	2 + 4	55.7	1.75	Absent	Animal
ce	1 + 3	40.5	2.5	Absent	Animal
ace	2 + 4	55.7	2.5	Absent	Animal
de	1 + 3	40.5	1.75	Present	Animal
ade	2 + 4	55.7	1.75	Present	Animal
cde	1 + 3	40.5	2.5	Present	Animal
acde	2 + 4	55.7	2.5	Present	Animal

Thirty-two shortening samples were prepared by using the requisite amount of the four emulsifiers or blends of two of the four monoglycerides. Each shortening was heated to 150°F., chilled, and plasticized in a laboratory-batch Votator under carefully-controlled conditions, tempered or not tempered as the design required, and the water-absorption, icing-volume, and cake-baking tests were performed. The data are summarized in Table XI.

Results and Conclusions

The data obtained from the shortening samples wherein the various treatment combinations had been incorporated were then subjected to statistical analysis, as previously described. Initially the analysis of variance was employed in which the Yates Method (6) was used to calculate the Effects and Mean Squares. The method is illustrated in Table XII.

In addition, a graphical method (7) was used which we have designated Half-Normal Graphical Analysis (Figure 2). This delineates those effects which are significant but does not give the sign of the effect, *i.e.,* whether the property being measured

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[1] Trade name of the HumKo Company, Memphis, Tenn.
[2] Trade name of Armour and Company, Chicago, Ill.

TABLE XI (a)

Summary of Results—Initial Experiment

Treatment combinations	Icing cc./100 g.	Water-absorption	130% White cake		Yellow-cake		Devil's food cake	
	Volume	Percentage	Volume, cc.	Score	Volume, cc.	Score	Volume, cc.	Score
(1)	143	503	1900	88	2560	99	2130	86
a	143	657	1900	90	2430	90	2150	88
b	144	603	1855	86	2500	97	2200	87
ab	144	774	2000	94	2500	97	2185	93
c	135	576	1975	93	2470	94	2140	87
ac	139	601	1900	87	2480	95	2240	95
bc	133	555	1960	91	2440	91	2225	91
abc	129	809	1850	86	2450	92	2245	96
d	141	477	1870	87	2485	96	2205	92
ad	154	584	1850	85	2565	99	2195	92
bd	141	450	1950	89	2425	89	2185	90
abd	144	564	1915	88	2500	97	2165	92
cd	136	724	1950	90	2400	87	2190	93
acd	139	557	1900	88	2480	95	2120	84
bcd	133	751	1950	90	2400	87	2195	92
abcd	132	869	2050	96	2410	88	2160	87

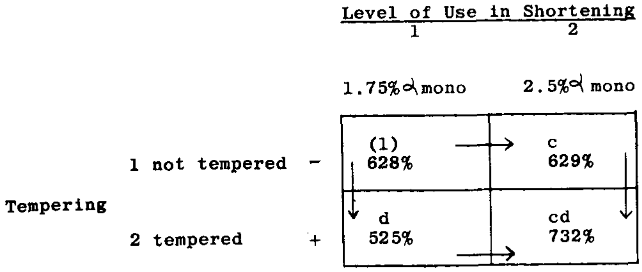
TABLE XI (b)

Summary of Results—Second Experiment

Treatment combinations	Icing volume	Water-absorption percentage
(1)	143.5	473.0
a	145.0	546.0
c	115.0	987.5
ac	106.5	992.0
d	141.5	553.5
ad	141.5	555.0
cd	112.0	994.0
acd	108.5	949.5
e	134.0	599.0
ae	131.0	629.5
ce	105.5	849.4
ace	99.5	556.5
de	137.5	584.5
ade	135.5	660.5
cde	105.0	926.0
acde	101.5	956.0

is improved in going from the low level to the higher level. Hence it is necessary that the graphical method be used in conjunction with or in addition to the analysis of variance.

In the case of water absorption it was found that the highest absorption was obtained when the emulsifier is at the higher level (2.5% *alpha* mono) in the shortening. There also was an interaction between the higher level of use and the presence of tempering. The diagram below serves to illustrate this interaction more clearly.



The diagram indicates the water-absorption values which might be expected under conditions extant in each square. Results from the experiment would indicate a decrease in water absorption when the shortening is tempered. However it is known that erratic results are obtained when the shortening is not tempered. However on going from the low level, where 1.75% α mono is added to the shortening, to the higher level, 2½% α mono, there is a significant improvement in water absorption, particularly when combined with tempering. The *alpha* mono content of the emulsifier, the I.V. of the emulsifier, or the shortening base stock does not have any significant effect upon the absorption characteristics of the shortening. These conclusions are based upon results at the 95% confidence level.

Where optimum icing-volume is desired, it was found that the best results could be obtained from a shortening containing the low level of addition of emulsifier, plus the use of a vegetable-base stock shortening. The use of the high level of addition or a meat-fat type of shortening had a significant effect

TABLE XII

Analysis of Water Absorption Data

Treatment Combination B	Percentage	Percentage less 500	(1)	(2)	(3)	(4)	Effect	Mean squares
(1)	473.0	—27.0	19.0	998.5	2050.5	3815	238.4	....
a	546.0	46.0	979.5	1052.0	1764.5	—119	—14.9	14,161
c	987.5	487.5	108.5	634.2	34.5	2614	326.8	6,832,996**
ac	992.0	492.0	943.5	1130.0	—153.5	—481	—60.1	231,361
d	553.5	53.5	228.5	77.5	1796.5	549	68.6	301,401
ad	555.0	55.0	406.0	—43.0	817.5	251	31.4	63,001
cd	994.0	494.0	245.0	—262.5	—114.5	337	42.1	113,569
acd	949.5	449.5	885.0	109.0	—366.5	303	37.9	91,809
e	599.0	99.0	73.0	960.5	53.5	—286	—35.8	81,796
ae	629.5	129.5	4.5	835.0	495.5	—188	—23.5	35,344
ce	849.4	349.5	1.5	177.5	—120.5	—979	—122.4	958,441*
ace	556.5	56.5	—44.5	640.0	371.5	—252	—31.5	63,504
de	584.5	84.5	30.5	—68.5	—125.5	442	55.3	195,364
ade	660.5	160.5	—293.0	—46.0	462.5	492	61.5	242,064
cde	926.0	426.0	76.0	—323.5	22.5	588	73.5	345,744
acde	959.0	459.0	33.0	—43.0	280.5	258	32.3	66,564

Significant level: 95% 1,070,404.

TABLE XIII

Summary of Results

Properties	Experiment average	Significant factors	Confidence limits (95%)
Water absorption	628	Level of addition, temp'g	±99
Icing volume	140.5	Level of addition	±3.9
White cake score	89	None	±3.5
White cake volume	1901	None	±102
Yellow cake score	94	Level of addition	±3.9
Yellow cake volume	2465	None	±88
Devil's food score	90	Mono content, tempering	±3.6
Devil's food volume	2182	None	±91

on the icing volume in a negative way. There was no interaction of factors, and the factors, *alpha* mono content of emulsifier, I.V. of emulsifier, and tempering showed no significant effects.

It is well known that maximum icing-volume is obtained with minimum or no monoglyceride added to the shortening stock. However, in order to obtain desirable icing characteristics, such as moisture retention, creaminess, and nonweeping, the use of some mono and diglycerides is indicated, but these properties are obtained with some reduction in icing volume.

In the ranges examined for the five factors, we were unable to show any significant effect on either the volume or over-all cake score of the 130% white cake, which is commonly used to evaluate shortenings and emulsifiers. In the case of the yellow cake the graphical decision method does indicate that the level of use of the monoglyceride in the shortening is significant. However, contrary to what might be expected in going from the low level, where 1.75% *alpha* mono is added to the shortening, to the higher level, where 2.5% *alpha* mono is added to the shortening, the sign of the effect is negative.

Again it is fairly well accepted by those in the prepared mix industry that as the level of monoglyceride in cake mix shortening is increased, there are two peaks. The first maximum in volume occurs somewhere around 2½% *alpha* mono, and the second slightly higher maximum at about 4 to 4½% *alpha* mono. Since we added sufficient emulsifier to give 2½% mono added, the shortening would analyze about 2.9 to 3% *alpha* mono. Apparently the levels of use were on either side of the initial maximum, which is quite peaked as compared to the second maximum.

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The Allergen Content of Castor Beans and Castor Pomace

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The allergen content of 10 varieties of decorticated, defatted castor beans, as determined by a serological method, ranged from 6.1 to 9.0%. This range of allergen content probably does not offer an encouraging prospect for the development of an allergen-free castor bean by plant breeding. The allergen content of samples of commercial castor pomace ranged from 0.092 to 4.2%. It is apparent from these results that some current commercial milling practices are capable of reducing significantly the allergen content of castor pomace.

THE CASTOR BEAN ALLERGEN is a nontoxic, unusually stable protein (1–5) that exhibits an extraordinary capacity to sensitize individuals exposed to small concentrations of the dust from castor beans or castor pomace (6–10). Allergic diseases caused by sensitivity to castor beans or castor bean by-products appear likely to become an increasingly serious problem as the growing, transportation, and processing of the beans become more widespread in this country (11,12).

Maximal utilization of castor pomace as a source of industrial proteins depends largely on the development of a feasible method for inactivation or elimination of the castor bean allergen. Previous endeavors (13,14) to inactivate the allergen by chemical and physical methods indicate that reactions drastic enough to destroy the allergen also destroy the useful proteins. Selective plant-breeding has been proposed (15) as a possible means of diminishing and ultimately eliminating the undesirable allergenic component from castor beans. The feasibility of such a program would depend upon the demonstration of a natural variation in concentration of the allergenic component in different varieties of castor beans. The purpose of the present study was to investigate the range of allergen concentration in different varieties of castor beans in order to supply this information. Also the allergen content of a few samples of commercial castor pomace was investigated to determine the effect on the allergenic components of different, current castor-bean milling practices.

The quantitative precipitin method developed by Heidelberger and Kendall (16) was used for determining the allergen content of castor beans. This method utilizes the normal immunological protective reaction of a live rabbit to an invasion of its tissues by a foreign protein. Injection of the foreign protein, called antigen, stimulates the production of some newly formed blood proteins, called antibodies. Antibodies exhibit the unique property of reacting spe-

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