products of the calculations are adjusted to a 100% basis and represent the percentages of the original S_3, S_2U, SU_2 and U_3.

ACKNOWLEDGMENT

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Application of Statistical Distribution Formulas to Triglycerides Originating in Tissues Having Regional Differences in Fatty Acid Composition[1]

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Abstract

Variations in triglyceride fatty acid composition are sometimes observed between different regions of the same fatty tissue in various plants and animals. When natural fats originate in such tissues, some error results if triglyceride distribution formulas are applied to an *average* fatty acid composition. An integral calculus technique has been developed to overcome this difficulty. This method has been applied to the random, restricted-random, and 1,3-random-2-random distribution hypotheses. The error resulting from such regional differences has been estimated for five natural fats originating in such heterogeneous tissues.

If the relative amounts but not the types of fatty acids vary with location, regional differences do not appreciably affect the use of statistical distribution formulas. In such cases, triglyceride composition may be predicted from the average fatty acid composition without significant error. If different types of fatty acids exist in different regions, however, these differences must be taken into account to avoid large errors.

Introduction

A NUMBER OF WORKERS have reported that morphologically-distinct fatty tissues in various plants and animals contain regional differences in fatty acid composition. Kartha (1) has reported substantial variations in the iodine values of fats from different locations in the almond, peanut, pistachio nut, Brazil nut, cashew nut, sapota seed, coconut, hazelnut, apricot seed, and arecanut. He observed extreme regional differences in the coconut where the iodine value varied from 7.9 to 46.0 according to the location of the sample. Galoppini and Lotti (2) have reported regional variations in the fatty acid compositions of the peanut, hazelnut, almond, and pine nut. In our own laboratory, we have observed regional variations in the fatty acid and triglyceride compositions of *Myrica carolinensis* fruit coat fat (3). The phenomenon is not restricted to plant fats. Hilditch and Zaky (4) have demonstrated the different fatty acid and triglyceride compositions of perinephric and external tissue fats from the same sheep.

[1] Presented at the AOCS meeting, Houston, 1965.

This accumulating evidence indicates that many of the fats being studied for chemical composition are not of homogeneous origin. Apparently many natural fats and oils are not "pure" fats but mixtures of many similar fats of slightly varying composition. If fatty tissue exhibits regional differences in fatty acid composition, then regional differences in triglyceride composition must also exist. These regional differences affect the applicability of the various triglyceride distribution hypotheses. This can be demonstrated by examining a hypothetical fat of mixed origin and composition.

Consider a 1:1 mixture of palm and palm kernel oils. The palm oil contains 0% and the palm kernel oil 54.6% lauric acid (Table VI). The lauric acid from the palm kernel oil cannot enter into any of the palm oil triglycerides as required by the even, random, restricted-random, or 1,3-random-2-random distribution hypotheses. Therefore, a mixture of two fats having different fatty acid compositions cannot follow statistical distribution rules exactly.

The above case is obviously an extreme example of how mixed fats can deviate from the triglyceride compositions predicted by distribution hypotheses. But the same principle still applies when a fat originates in a morphologically-distinct tissue having smaller regional differences in fatty acid composition. It is impossible for present distribution formulas to exactly describe the triglyceride composition of the resultant mixed fat.

For the interpretation of our experimental results on *Myrica carolinensis* fruit coat fat, we have developed an integral calculus technique which does allow statistical distribution hypotheses to be accurately applied to fats originating in tissues having regional differences in fatty acid composition. This paper describes the new method and its application to the random, restricted-random, and 1,3-random-2-random hypotheses. Five natural fats originating from heterogeneous sources have been examined using this technique.

It should be emphasized that *this paper does not deal with the relative merits of the various triglyceride distribution hypotheses discussed.* We describe only a technique for applying these hypotheses to fatty tissues having regional differences in fatty acid composition.

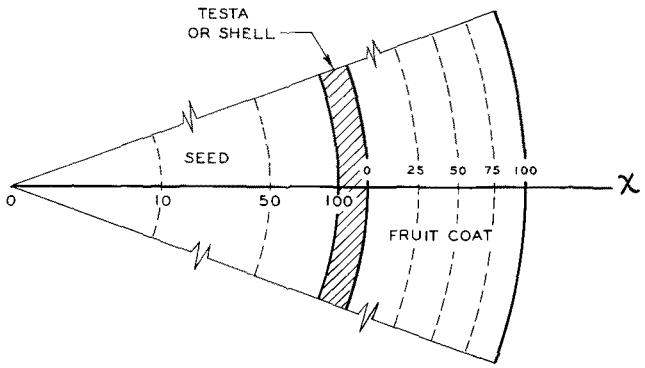

FIG. 1. One-dimensional positional axes for a model spherical oilseed covered by a spherical fruit coat. The 0–100 scales are measured in units of mole percent total triglyceride and not in distance from the center of the seed or inner surface of the fruit coat. If a separate embryo is present, it is assumed to be at the center of the seed.

General Method

The first step in applying distribution hypotheses to fats originating in tissues having regional differences in fatty acid composition is to experimentally determine what regional differences exist. The original seed or fruit is divided into small sections or layers and the fat is separately extracted from each fraction. The triglycerides are isolated, and their fatty acid composition is determined by gas-liquid chromatography (GLC). The extreme sensitivity of GLC permits the analysis of very small fractions from seeds or fruits of modest size, so that regional differences can be accurately defined.

Once the regional differences have been determined, the fatty acid composition can be expressed mathematically as a function of location. This is done by setting up positional axes in the seed or fruit coat. Figure 1 illustrates such positional axes in a model spherical oilseed which is covered by a spherical fruit coat. (In some cases such as the peanut, there is no fruit coat layer and only the seed axis of Figure 1 is applicable.) The specific fatty tissue is thought of as a series of concentric spheres, each having a uniform fatty acid composition. The position of each sphere can be located along a radial axis X measured from 0 at the center of the seed (or inner surface of the fruit coat) to 100 at the outer surface of the seed (or fruit coat). This 0–100 scale is measured in units of mole percent total triglyceride, not in distance from the center of the seed (or inner surface of fruit coat). If a separate embryo is present, it is convenient to assume it is located at the center of the seed. If the seed or fruit coat has a nonspherical shape, or if the fatty acid composition of each concentric sphere is not uniform, then similar Y and Z axes may be necessary to completely describe the compositions at all locations. Calculations are greatly simplified, however, when only one positional axis is used. Only this monoaxial case will be considered here.

TABLE I
Application of 1,3-Random-2-Random Distribution Formulas to *Myrica carolinensis* Fruit Coat Fat

Triglyceride	Calculated using integration formulas		Calculated from average fatty acid composition
	Range	Total	
	mole %	mole %	mole %
PPP	26.7–70.8	46.6	45.6
PMP	17.6–30.2	25.4	26.3
MPP	6.7–16.3	13.7	14.8
MMP	1.7–18.5	9.0	8.5
MPM	0.2– 2.5	1.2	1.2
MMM	tr– 2.8	0.9	0.7
PPSt	0.7– 1.5	1.1	1.1
PStP	0.6– 0.9	0.7	0.7
PMSt	0.4– 0.8	0.6	0.6
Others	....	0.8	0.5

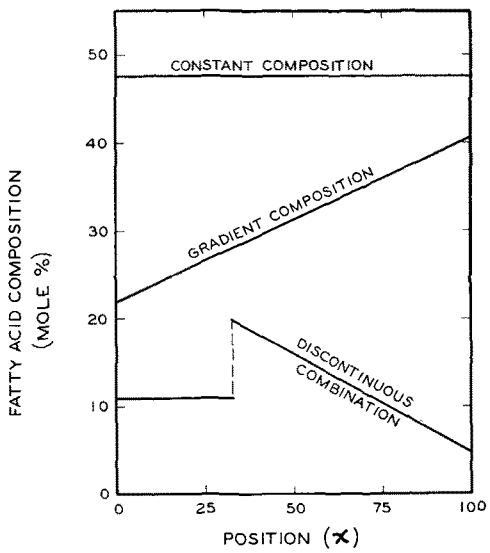

FIG. 2. Possible one-dimensional fatty acid profiles that can occur in an oilseed or fruit coat.

When appropriate positional axes have been established in the oilseed or fruit coat, experimental fatty acid compositions can be graphed as a function of position. Three possible fatty acid profiles can result (see Fig. 2). A horizontal straight line is obtained if the mole percent of a fatty acid is the same in all parts of the seed. A gradient composition curve results if fatty acid composition varies with location. A discontinuous combination curve occurs when there is an abrupt change of fatty acid composition at one particular point. The latter case usually indicates a change from one morphologically-distinct tissue to another, such as between the embryo and cotyledon of the peanut.

Once the experimental fatty acid profiles have been established, the resultant curves can be expressed as mathematical equations. The percent of each fatty acid is now known as a function of x, the location. The original seed or fruit coat is theoretically divided into an infinite number of concentric spheres, each having a thickness dx and a constant fatty acid composition. The appropriate triglyceride distribution formula can then be applied to each separate dx sphere and the result integrated from $x = 0$ through $x = 100$. This standard integration procedure will accurately sum up all the triglycerides predicted for each individual dx sphere. The final result is an accurate prediction of the total triglyceride composition of the seed taking regional differences in fatty acid composition into account.

This general procedure is illustrated in detail by the five specific applications described below.

Specific Applications

Myrica carolinensis Fruit Coat Fat

Harlow et al. (3) have recently described the regional differences in fatty acid composition in *Myrica carolinensis* fruit coat fat. Figure 3 shows a fatty acid profile graph constructed from their lipase hydrolysis data. Mathematical equations were derived for the mole percent of 14:0, 16:0, and 18:0[1] at the

[1] The following shorthand designations will be used for fatty acids and triglycerides: M = 14:0 = myristic acid; P = 16:0 = palmitic acid; St = 18:0 = stearic acid; O = 18:1 = oleic acid; L = 18:2 = linoleic acid; U = unsaturated fatty acid; S = saturated fatty acid; PLO = 1-palmito-2-linoleo-3-olein; MMP = 1,2-dimyristo-3-palmitin; SSS = trisaturated triglyceride; etc.

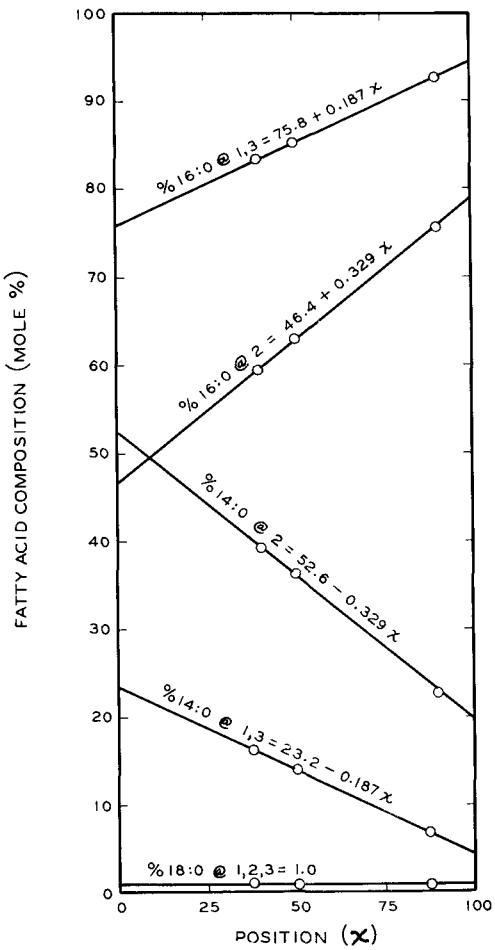

FIG. 3. Fatty acid profile of the *Myrica carolinensis* fruit coat (3).

1,3-positions and at the 2-positions of the triglycerides. This information was used to predict the triglyceride composition of *M. carolinensis* fruit coat fat based on the 1,3-random-2-random distribution hypothesis of VanderWal (5) and Coleman and Fulton (6).

The amount of MMP triglyceride predicted by the 1,3-random-2-random distribution hypothesis was calculated from the equation:

$$\%\text{MMP} = \frac{2}{10^4}\left(\begin{matrix}\%14:0 \text{ at} \\ 1,3\text{-positions}\end{matrix}\right)\left(\begin{matrix}\%14:0 \text{ at} \\ 2\text{-position}\end{matrix}\right)\left(\begin{matrix}\%16:0 \text{ at} \\ 1,3\text{-positions}\end{matrix}\right)$$

Substituting in the mathematical expressions for varying fatty acid composition and integrating from x = 0 through x = 100, we obtained:

$$\%\text{MMP} = \frac{2}{10^6}\int_0^{100} (23.2 - 0.187x)(52.6 - 0.329x)(75.8 + 0.187x)\,dx$$

Note that multiplying the right side of the equation by dx and integrating was equivalent to multiplying by 100. Therefore, the right side was also divided by 100 to compensate. This integral was evaluated to obtain:

$$\%\text{MMP} = \frac{2}{10^6}\int_0^{100} (92.500 - 1095.95x + 1.3967x^2 + 0.0115048x^3)\,dx$$
$$= 9.0\%$$

Similar calculations were carried out for all possible triglycerides present in amounts greater than 0.5 mole %.

A parallel computation of triglyceride composition was made using the *average* fatty acid composition of the total fruit coat triglycerides, i.e. not allowing for regional differences in fatty acid composition. Both

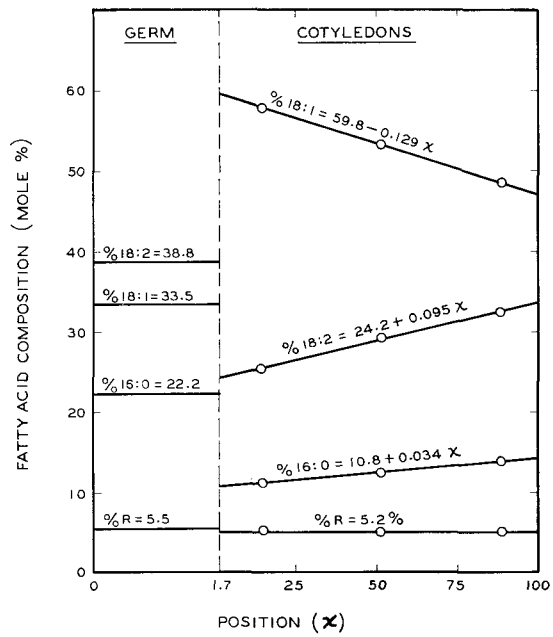

FIG. 4. Fatty acid profile of the peanut (2). R represents a combination of all fatty acids other than 16:0, 18:1, and 18:2.

results are compared in Table I along with the range of triglyceride compositions which would be predicted in various parts of the fruit coat based on data in Figure 3. Unexpectedly, the triglyceride compositions predicted using integration formulas and those predicted using average values were remarkably close. Even though the amount of PPP varied from 26.7 mole % at the inside surface of the fruit coat to 70.8% at the outside surface, the integration technique predicted 46.6% and the average technique 45.6% PPP in the total triglycerides. Values for other individual triglycerides were similarly close.

Peanut Oil

Galoppini and Lotti (2) have reported the regional differences in fatty acid composition found in the peanut. Figure 4 shows a fatty acid profile graph constructed from their data. Regional variations in mole percent 16:0, 18:1 and 18:2 were present, but the remaining fatty acids (combined into the term R) were approximately constant throughout the fatty tissue. Differences between the germ and cotyledon

TABLE II

Application of Random Distribution Formulas to Peanut Fat

Triglyceride[a]	Calculated using integration formulas		Calculated from average fatty acid composition
	Range	Total	
	mole %	mole %	mole %
OLO + OOL	13.1–26.0	24.3	24.5
OOO	3.8–21.2	15.1	14.8
LOL + LLO	10.6–16.0	13.4	13.5
PLO + POL + OPL	9.5–17.3	11.7	11.8
OPO + OOP	7.5–11.6	10.5	10.7
ROL + RLO + ORL	4.3– 4.9	4.8	4.8
ORO + ROO	1.9– 5.5	4.4	4.4
LPL + PLL	1.9–10.0	3.4	3.2
LLL	1.5– 5.8	2.6	2.5
POP + PPO	2.1– 5.0	2.5	2.6
RPO + POR + PRO	2.0– 3.4	2.1	2.1
PLP + PPL	0.9– 5.7	1.5	1.4
LRL + RLL	0.9– 2.5	1.3	1.3
RPL + PLR + PRL	0.8– 2.8	1.2	1.2
ROR + RRO	0.3– 0.5	0.4	0.4
PRP + RPP	0.2– 0.8	0.3	0.3
PPP	0.1– 1.1	0.2	0.2
RLR + RRL	0.2– 0.4	0.2	0.2
RPR + RRP	0.1– 0.2	0.1	0.1
RRR	.....	tr	tr

[a] R represents a combination of all fatty acids other than palmitic, oleic, and linoleic.

TABLE III

Application of Restricted-Random Distribution Formulas to "Mutton Tallow" Triglycerides Originating in Tissues of Different Composition

	Sheep tissue fats		"Mutton Tallow"	
	External	Perinephric	Calculated from 7:3 mixture of external and perinephric triglycerides	Calculated from S and SSS of 7:3 mixture of external and perinephric fats
	mole %	mole %	mole %	mole %
	Experimental		Calculated	
S	47.7	56.9	.....	50.5
SSS	5.5	14.4	8.2	8.2
	Calculated			
SUS + SSU	44.5	48.8	45.7	45.8
USU + SUU	37.6	29.9	35.5	35.3
UUU	12.4	6.9	10.6	10.7

fats were represented by discontinuous curves. Mathematical equations were derived to describe the regional variations in fatty acid composition in a spherical model peanut with the embryo at the center. This information was used to predict the triglyceride composition of peanut oil using the random distribution hypothesis (7).

The amount of POL + PLO + OPL triglyceride predicted by random distribution of fatty acids was calculated from the equation:

$$\%(POL+PLO+OPL)=\frac{6}{10^4}(0.017)\left(\frac{\%\ 16:0}{\text{in germ}}\right)\left(\frac{\%\ 18:1}{\text{in germ}}\right)\left(\frac{\%\ 18:2}{\text{in germ}}\right)$$
$$+\frac{6}{10^4}(0.983)\left(\frac{\%\ 16:0\text{ in}}{\text{cotyledons}}\right)\left(\frac{\%\ 18:1\text{ in}}{\text{cotyledons}}\right)\left(\frac{\%\ 18:2\text{ in}}{\text{cotyledons}}\right)$$

Two terms were included in the right hand side of the equation to account for the different compositions of the germ and cotyledon fats. Substituting numerical values for germ fatty acid composition and mathematical expressions for varying cotyledon fatty acid composition, and integrating the cotyledon terms from x = 1.7 to x = 100, we obtained:

$$\%(POL+PLO+OPL)=\frac{6}{10^4}(0.017)(22.2)(33.5)(38.8)$$
$$+\frac{6}{10^6}\int_{1.7}^{100}(10.8+0.034x)(59.8-0.129x)(24.2+0.095x)dx$$

This equation was solved to obtain:

$$\%(POL+PLO+OPL)=$$
$$0.294+\frac{6}{10^6}\int_{1.7}^{100}(15{,}629+76.84x-0.0453x^2-0.000417x^3)dx$$
$$=0.294+11.370=11.7\%$$

Similar calculations were carried out for all possible triglycerides containing P, O, L, and R.

A parallel computation of triglyceride composition was made using the *average* fatty acid composition of the total fat. Both results were compared (Table II) along with the range of triglyceride compositions which would be predicted in various parts of the peanut based on the data in Figure 5. Once again, the triglyceride composition predicted using integration formulas and that predicted using average fatty acid values were quite close. Even though the amount of 000 varied from 3.8 mole % in the germ to 21.2% at the inner surface of the cotyledon, the integration technique predicted 15.1% and the average technique 14.8% 000 in the total triglycerides. Values for other individual triglycerides were even closer together.

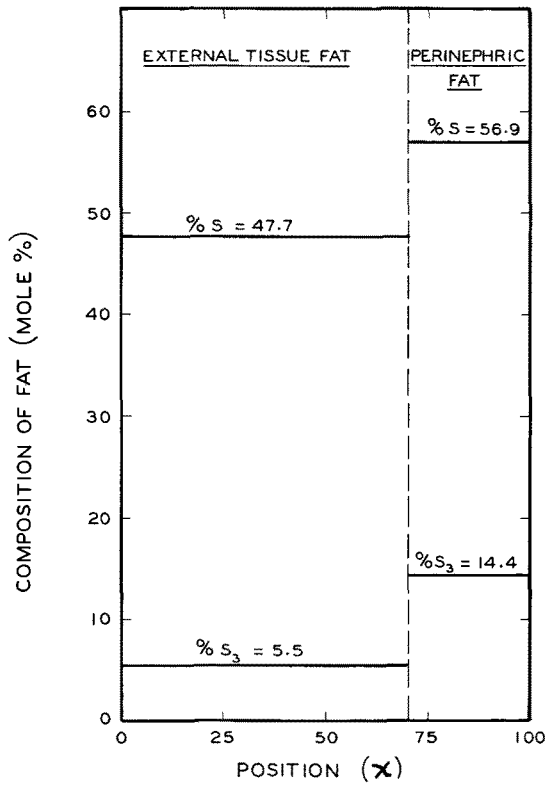

FIG. 5. Fatty acid and saturated triglyceride profile of the external and perinephric tissues from the same sheep (4). Fats from these two tissues were combined to produce a hypothetical mutton tallow.

Mutton Tallow

Hilditch and Zaky (4) have demonstrated the different fatty acid compositions of perinephric and external tissue fats from the same sheep. We combined these two fats in the same ratio in which they were present in the sheep to form a hypothetical mutton tallow having the compositional profile shown in Figure 5. Since tallow is normally a mixture of several different tissue fats, this hypothetical case was a fair approximation of a commercial mutton tallow. The compositional profile was expressed in terms of mole percent saturated fatty acids (% S) and mole percent saturated triglycerides (% SSS) so that Kartha's restricted-random distribution formulas (8) could be applied. No integration was necessary in this calculation since the separate fats were assumed to have

TABLE IV

Fatty Acid Composition of a Mixture of Two Different Palm Oils [a] (mole %)

Fatty acid	Total triglycerides			1,3-Positions			2-Position		
	Palm oil A (11)	Palm oil B (12)	1:1 Mixture of palm oils A and B	Palm oil A	Palm oil B	1:1 Mixture of palm oils A and B	Palm oil A (11)	Palm oil B (12)	1:1 Mixture of palm oils A and B
14:0	0.3	1.7	1.0	0.4	2.2	1.3	.....	0.7	0.3
16:0	40.6	44.3	42.5	56.6	60.5	58.6	8.6	11.9	10.3
18:0	4.6	5.2	4.9	6.7	7.3	7.0	0.5	0.9	0.7
18:1	46.6	36.8	41.7	32.7	24.0	28.3	74.5	62.5	68.5
18:2	7.9	12.0	9.9	3.6	6.0	4.8	16.4	24.0	20.2

[a] All components present in 0.2 mole % or less were omitted. Compositions were normalized to 100.0%.

TABLE V

Application of 1,3-Random-2-Random Distribution Formulas to a Mixture of Two Different Palm Oils

Triglyceride	Composition predicted by 1,3-random-2-random distribution of fatty acids			1:1 Mixture of predicted triglycerides for palm oils A and B
	Palm oil A	Palm oil B	1:1 Mixture of palm oils A and B	
	mole %	mole %	mole %	mole %
POP	23.9	22.9	23.5	23.4
POO	27.6	18.2	22.7	22.9
PLP	5.3	8.8	6.9	7.0
PLO	6.1	7.0	6.7	6.5
OOO	8.0	3.6	5.5	5.8
POSt	5.7	5.5	5.6	5.6
POL	3.0	4.5	3.9	3.8
PPP	2.8	4.4	3.5	3.6
PPO	3.2	3.5	3.4	3.3
StOO	3.3	2.2	2.7	2.7
OOL	1.8	1.8	1.9	1.8
PLSt	1.2	2.1	1.7	1.7
OLO	1.8	1.4	1.6	1.6
PLL	0.7	1.7	1.1	1.2
MOP	0.3	1.7	1.0	1.0
PPSt	0.7	1.1	0.8	0.9
OPO	0.9	0.7	0.8	0.8
StLO	0.7	0.8	0.8	0.8
Others	3.0	8.1	5.9	5.6

no regional variations in fatty acid composition within their respective tissues.

The restricted-random triglyceride compositions predicted for the external tissue fat, the perinephric tissue fat, and a 7:3 mixture of the two fats were calculated (Table III). The triglyceride composition of a 7:3 mixture of the predicted triglycerides for external and perinephric tissue fats was also computed (Table III). The triglyceride composition obtained by mixing predicted component triglycerides was almost identical with that calculated from the average % S and % SSS of the mixture.

We have also developed integrated restricted-random distribution formulas for use with fats originating in tissues having regional differences in fatty acid composition. Unfortunately, these equations were so complex that integration calculations were too lengthy to be practical.

$$SSS = S_3 = f(x) \text{ determined experimentally}$$

$$SUS + SSU = \frac{1}{100}\int_0^{100} [300S^2 - 2S^3 - S_3 + 3a]\,dx$$

$$USU + SUU = \frac{1}{100}\int_0^{100} [3S(100 - S)^2 - 3a + 3b]\,dx$$

$$UUU = \frac{1}{100}\int_0^{100} [(100 - S)^3 - 3b]\,dx$$

where:

$$S = g(x) \text{ determined experimentally}$$

$$3a = \frac{2S^4 - 2SS_3}{S + 100}$$

$$3b = \frac{(100 - S)(S^3 - S_3)}{S + 100}$$

Values for S, S_3, SUS + SSU, USU + SUU, and UUU are expressed in mole fraction units.

Palm Oil

It is well known that factors such as climate, plant variety, soil, etc., have a marked influence on the fatty acid composition of natural fats (9). It has even been shown that different soybeans from the same plant have different fatty acid compositions (10). Most of the commercial fat samples examined for triglyceride composition have a diverse origin similar to that already described for fats from tissues having regional differences in fatty acid composition. For example, two palm oils originating from different plant varieties and having different fatty acid compositions are often mixed together in the tanks of an oil extraction plant. To determine if such commercial mixing practices could affect the application of triglyceride distribution hypotheses, a mixture of two different palm oils was examined.

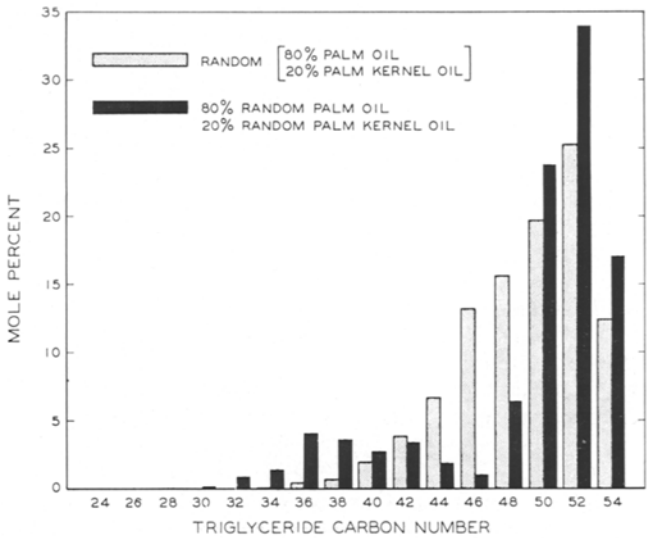

FIG. 6. Carbon number distribution of triglycerides in a randomized 4:1 mixture of palm and palm kernel oils and in a 4:1 mixture of randomized palm oil with randomized palm kernel oil.

Lipase hydrolysis results on palm oil A containing 46.6 mole % 18:1 have been reported by Savary and Desnuelle (11). Lipase hydrolysis results on palm oil B containing 36.8% 18:1 have been reported by Jurriens et al. (12). These data are given in Table IV. The lipase hydrolysis data from a hypothetical 1:1 mixture of these two oils were also calculated and recorded in Table IV.

The triglyceride compositions of palm oil A, palm oil B, and their 1:1 mixture were predicted from the data in Table IV using the 1,3-random-2-random distribution hypothesis (5,6). The triglyceride composition of the 1:1 mixture was also calculated by averaging the predicted triglyceride compositions of palm oils A and B. These results are shown in Table V. The triglyceride composition obtained by mixing predicted component triglycerides is very close to that calculated from the lipase hydrolysis results on the mixture itself.

Mixture of Palm and Palm Kernel Oils

In the four systems of mixed fats examined above, the same fatty acids were present in all parts of the fatty tissue. Only the relative amounts changed. It is also theoretically possible for the type of fatty acids present to vary with location. This often occurs if a natural fat is extracted from two or more morphologically-distinct tissues. To determine if such regional differences in the types of component fatty acids could affect the application of triglyceride distribution hypotheses, a total extract from the palm fruit (i.e., a mixture of palm and palm kernel oils) was examined.

Literature values for the fatty acid compositions of

TABLE VI

Fatty Acid Composition of a Mixture of Palm and Palm Kernel Oils

Fatty acid chain length	Palm oil (11)	Palm kernel oil (13)	4:1 Mixture of palm and palm kernel oils
	Mole %	Mole %	Mole %
8	.....	4.4	0.9
10	.....	4.9	1.0
12	.....	54.6	10.9
14	0.3	16.1	3.5
16	40.6	6.8	33.8
18	59.1	13.2	49.9

palm and palm kernel oils are given in Table VI. The fatty acid composition of a hypothetical 4:1 mixture of these two oils (their approximate molar ratio in the total palm fruit [14]) was also calculated and recorded in Table VI.

The carbon number distribution of triglycerides for palm oil, palm kernel oil, and their 4:1 mixture was calculated from the data in Table VI using the random distribution hypothesis (7). The carbon number distribution of the 4:1 mixture was also calculated from the predicted triglyceride compositions of the original palm and palm kernel oils. Figure 6 compares the carbon number distribution of triglycerides in the randomized mixture and in the mixture of the two random oils. It is very clear that the two predicted triglyceride compositions are very different.

Discussion

The integration technique described above now makes it possible to accurately apply triglyceride distribution hypotheses to natural fats originating in tissues having regional differences in fatty acid composition. The accuracy of the integration method is limited only by the experimental accuracy with which such regional differences can be defined. Although only one-dimensional regional differences have been treated here, the technique is inherently applicable to three-dimensional differences. In the latter case, however, a more complicated mathematical treatment is required, so that simplification to a one-dimensional model is often desirable.

When the relative amounts but not the types of fatty acids vary with location, the triglyceride composition can be predicted from the average fatty acid composition without introducing appreciable error. Discrepancies between results from the integration technique and results based on average fatty acid composition are less than the experimental error in current triglyceride analysis procedures. Where different types of fatty acids exist in different regions, however, these differences must be taken into account to avoid large errors. These conclusions apply to both intra- and interseed differences in fatty acid composition.

The five fats and three distribution hypotheses examined here were chosen as typical examples, but they do not necessarily cover all possible cases. They are meant to serve only as a guide showing how regional differences can be handled when distribution hypotheses are tested against experimental results.

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