The Component Glycerides of Soya Bean Oil and of Soya Bean Oil Fractions

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THE glyceride structure of soya bean oil has hitherto only been studied by indirect methods. Japanese workers (1) isolated various crystalline bromo-additive products of mixed glycerides from the oil which indicated the presence, *inter alia*, of oleodilinolein, linoleodiolein, oleolinoleolinolenin, some linoleolinolenins, and oleodipalmitin. Hilditch and Jones (2) showed that the completely hydrogenated oil only contains about 75% of tristearin whence it follows that all saturated acids other than stearic must be in combination with two unsaturated acids in mixed triglycerides and, further, that it is unlikely that either of the major component unsaturated acids (oleic 25-30%, linoleic 55-50%) are present in any appreciable quantity in the form of the simple triglycerides — triolein or trilinolein. It, therefore, seemed opportune to supplement these general indications that the "even distribution" rule was followed in soya bean oil by determining the chief component glycerides of this somewhat important oil by resolving it into a number of fractions by crystallisation from acetone at temperatures from —60° upwards and determining the component acids present in each of the fractions so obtained.

In recent years it has been proposed to use low-temperature solvent separation on a technical scale as a means of preparing from soya bean (and other) oils a fraction superior in "drying" qualities to the whole oil, and products of this kind are coming into the market in the United States; thus in one instance it has been stated that soya bean oil of iodine value 135 yields 30% of a paint oil with iodine value 162 and 67.5% of an "edible oil" fraction with iodine value 125. In 1943 Bull and Wheeler (3) studied the crystallisation of soya bean oil from a number of solvents at from —75° to 15° and stated that a high ratio of solvent (acetone) to oil, at a very low temperature, gave the best results. From an oil of iodine value 132.5, not more than 30-60% of most soluble fractions with iodine values varying from 155-145 were obtained although with a very high solvent ratio small yields of material of iodine value 165 were isolated. Using liquid phase partition with methyl alcohol at 25°, Kleinsmith and Kraybill (4) obtained unsaturated fractions of iodine value 140-146 from a soya bean oil of iodine value 132.5. The data obtained in the course of the present study of the glyceride composition of soya bean oil serve at the same time to indicate the component acids and glycerides present in soluble and separated fractions of soya bean oil which are comparable with those referred to in the literature to which reference has just been made.

Experimental

The soya bean oil examined (kindly provided by J. Bibby and Sons, Ltd.) was a refined specimen very closely resembling in analytical characteristics that studied in 1939 by Hilditch and Jasperson (5); it had saponification equivalent 290.6, iodine value 132.6, and contained 0.2% of free fatty acid (as oleic) and 0.5% of unsaponifiable matter.

Component acids of the whole oil. Since the values obtained by summation from the component acids as determined for each of the six glyceride fractions into which the oil was separated involve the possibility of cumulative experimental errors during the numerous analytical operations of somewhat greater magnitude than those in a direct analysis of the mixed fatty acids of the whole oil, the latter operation was also carried out by the procedure given by Gunstone and Hilditch (6). The soya bean oil mixed fatty acids (227.2 g.) were first crystallised from acetone (10 cc. per g.) at —60° for five hours, when 65.5 g. (D, iodine value 181.6) remained in solution. The separated acids (161.7 g.) were next crystallised from ether (10 cc. per g.) at —40° for five hours, when 35.7 (iodine value 20.0) separated, leaving 126.0 g. in solution. The latter acids were further crystallised from ether (10 cc. per g.) at —60°, when 99.2 g. (C, iodine value 163.5) remained in solution and 26.8 g. (iodine value 102.3) were deposited; the 35.7 g. which had separated at —40° were recrystallised under same conditions, when 30.2 g. (A, iodine value 1.8) separated, leaving 5.5 g. (iodine value 115.6) in solution. The latter acids and the acids deposited from ether at —60° were united to form fraction B (32.3 g., "soluble in ether at —40°", iodine value 104.5). Linolenic and linoleic acids were determined in the mixed acids of fractions B, C, and D spectroscopically after isomerisation with alkali [*loc cit.* (6)], and all four groups were converted into their methyl esters which were separated by fractional distillation in the usual manner. The final composition of the soya bean oil fatty acids, as thus determined, is shown in Table I.

The final values in Table I are within 1 unit per cent of those recorded by Hilditch and Jasperson (5) for a similar specimen except that their figures for oleic and linolenic acids (based on thiocyanometric analyses) are respectively 28.9 and 6.5% (wt.). It has recently been shown (11) that the spectrophotometric determination of linolenic acid may probably give somewhat higher figures than the true values when the proportion of the latter acid is not large; the true value in this instance is probably somewhat lower than that now recorded and somewhat higher than that obtained by thiocyanometric analysis.

Component glycerides of the soya bean oil. The oil (266.4 g.) was crystallised for five hours from acetone (10 cc. per g.) at —60°, the portion deposited being similarly crystallised at —50°, and so on at —40° and —30°. The portions left successively in solution at —60° (F), —50° (E), and —40° (D) were examined

TABLE I.

Component Acids of Soya Bean Oil.

Fraction		g.	%	Iodine value
A	Insoluble in ether at —40°	30.2	13.3	1.8
B	Soluble in ether at —40°	32.3	14.2	104.5
C	Soluble in ether at —60°	99.2	43.7	163.5
D	Soluble in acetone at —60°	65.5	28.8	181.6

Component acids (Increments % wt.)	A (13.3%)	B (14.2%)	C (43.7%)	D (28.8%)	Total	% (wt.) (excluding unsaponifiable)	% (mol.)
Myristic (and lower)	.....	.....	.....	0.4	0.4	0.4	0.6
Palmitic	9.0	1.4	0.1	0.1	10.6	10.6	11.5
Stearic	1.9	0.5	.....	.....	2.4	2.4	2.4
Saturated C_{20}, C_{22}, C_{24}	2.2	0.2	.....	.....	2.4	2.4	2.1
Hexadecenoic	0.1	0.1	0.8	.....	1.0	1.0	1.1
Oleic	0.1	8.2	10.6	4.5	23.4	23.5	23.1
Linoleic	.....	3.5	29.9	17.5	50.9	51.2	50.7
Linolenic	.....	0.3	2.2	5.9	8.4	8.5	8.5
Unsaponifiable	.....	.....	0.1	0.4	0.5	.....	.....

separately, as was that (A) deposited at —30°. The portion left in solution at —30°, however, was again crystallised from acetone (10 cc. per g.) at —40°, when a further deposit (B) was obtained, leaving more material (C) in solution at —40°.

The mixed fatty acids from all six glyceride fractions were separately determined. Those from fractions E and F were analysed spectrophotometrically without preliminary resolution; those from C and D were first resolved by crystallisation from ether at —60°, followed only by spectrophotometric analysis of each group of acids so obtained. The acids from fraction B were similarly first separated by ether at —60°, and were then determined by the spectroscopic procedure coupled with ester-fractionation, while the acids from fraction A were first crystallised from ether at —40°, both insoluble and soluble portions being methylated and analysed by ester-fractionation, with spectroscopic determination of linolenic and linoleic acids in the soluble portions (only oleic acid being present in the acids from A insoluble in ether at —40°).

The proportions and characteristics of the glycerides in fractions A-F of the oil, together with their component acids, are given in Table II.

Fraction F, from its iodine value and appearance, evidently contained all of the small proportion of the original oil which had undergone partial oxidation during storage. Its component acids were therefore assumed originally to have been of the same composition as those in fraction E.

Discussion

Inspection of Table II at once shows that the individual fatty acids are distributed extremely widely or evenly throughout all the triglycerides in the oil. Linolenic acid, although present to the greatest extent in the more unsaturated and soluble fractions, nevertheless still forms 6% of the acids in the most saturated and sparingly soluble fraction. Conversely, the saturated acids are concentrated in the most sparingly soluble fractions A (26%) and B (20.5%), but in the most unsaturated fractions their proportion does not fall much below 10% of the total component acids.

TABLE II.

Soya Bean Oil Fractions From Acetone.

	A	B	C	D	E	F
Soluble at	(Insoluble)	(Insoluble)	—40°	—40°	—50°	—60°
Weight (g.)	95.5	71.3	47.8	27.6	13.8	10.4
Iodine value	114.3	131.7	150.6	152.8	163.7	148.6
Saponification equivalent	289.4	288.3	289.0	292.7	294.7	317.3
Glycerides: % (wt.)	35.8	26.8	17.9	10.4	5.2	3.9
% (mol.)	36.0	27.0	18.0	10.3	5.1	3.6
Component Acids (% wt.)						
Myristic (or lower)	0.2	0.7				
Palmitic	15.6	16.7	11.5	9.5	7.2	7.2
Stearic	6.1	2.7				
Saturated C_{20}, C_{22}, C_{24}	3.3	0.4				
Hexadecenoic	1.8	3.2	19.5	20.2	15.9	15.9
Oleic	25.4	16.3				
Linoleic	41.2	51.1	57.1	56.9	59.2	59.2
Linolenic	6.4	8.9	11.9	13.4	17.7	17.7
Component Acids (% mol.)						
Myristic (or lower)	0.3	0.8				
Palmitic	16.9	18.0	12.4	10.3	7.8	7.8
Stearic	6.0	2.6				
Saturated C_{20}, C_{22}, C_{24}	2.9	0.4				
Hexadecenoic	1.9	3.5	19.2	19.8	15.7	15.7
Oleic	24.9	15.9				
Linoleic	40.7	50.0	56.5	56.5	58.8	58.8
Linolenic	6.4	8.8	11.9	13.4	17.7	17.7

Again, the relative percentage proportions (wt.) of the saturated acids in the whole oil and in the least soluble fractions A and B are as follows:

Saturated acids	Whole oil	Fraction A	Fraction B
	(% wt.)	(% wt.)	(% wt.)
Myristic (or lower)	3	1	3
Palmitic	67	62	82
Stearic	15	24	13
Saturated-C_{20}, C_{22}, C_{24}	15	13	2

As would be expected, since there is no evidence that more than one saturated acyl group is present in any one triglyceride molecule, stearic and higher saturated acids tend to concentrate in the least soluble glycerides in fraction A, whilst in the succeeding fraction B palmitic acid forms over 80% of the saturated acids, and this is probably almost the only saturated acid present in the glycerides of the more soluble glycerides (fractions (C, D, E, F).

It may be added here that individual saturated acids present in some of the ester-fractions or acid groups isolated during our examination of the more saturated portions of the acids from some of the glyceride fractions were also investigated by adsorption methods, using the "frontal analysis" technique described by Claesson (10). For example, the acids in the final distilled ester-fraction (AA6) and the residual esters (AA7) of the mainly saturated acids (obtained by crystallisation from ether at —40°) of the mixed acids of glyceride fraction A (Table II), and the acids (DA) of glyceride fraction D which were insoluble in ether at —60°, were adsorbed on activated carbon from 0.3-0.4% solutions in absolute ethyl alcohol with the following results:

	Acids AA6		Acids AA7		Acids DA	
Mean molecular wt.	283.1		307.2		266.7	
Iodine value...	26.1		7.9		75.8	
	Adsorption	Calc.	Adsorption	Calc.	Adsorption	Calc.
	%	%	%	%	%	%
Myristic	....	....	....	....	20	....
Palmitic	....	1	....	....	24	54
Stearic	75	70	43	7	10	....
Arachidic	....	....	29	84	....	....
Behenic	....	....	23	....	....	....
Lignoceric	....	....	....	....	....	....
Oleic (and polyethenoid C_{18}	25	29	5	9	46	46

The data calculated in the usual manner from the equivalents and unsaturated acid contents of the three examples are added in each case in the columns headed "calc." The amounts of the three fractions AA6, AA7, and DA represented only very small proportions of the whole soya bean oil (namely, AA6, 1.7%; AA7, 1.4%; and DA, 1.3% of the total fat).

In adsorption "frontal" analyses palmitic and unsaturated C_{18} acids are evaluated together and the latter must be determined from iodine values and, where necessary, spectrophotometric analysis after alkali-isomerisation. For small proportions of unsaturated acids the adsorption method tends to give low values for the latter. In determining higher saturated fatty acids, the adsorption technique of Claesson (*loc. cit.*) should be a useful addition to ester-fractionation procedure: a) when the amount of acids available is too small for resolution by low-temperature crystallisation and subsequent conversion to esters, or b) when the saturated acids present in an ester-fraction are suspected to contain more than two members of the homologous series of natural saturated higher aliphatic acids.

The increments (% mol.) of saturated, oleic (including the small amounts of hexadecenoic acid recorded), linoleic, and linolenic acids in the amount of each of the glyceride fractions A to F, corresponding with 100 mol. of the original oil, can be calculated (Table III) from the data recorded in Table II. Probable combinations of these four groups of acids in mixed glycerides can also be given. Thus, within fairly narrow limits, it may be assumed that the solvent separation has given binary mixtures of glycerides consisting of tri-unsaturated and mono-saturated di-unsaturated glycerides since no indication has been forthcoming in the most saturated fraction A of the presence of any mono-unsaturated or of any fully-saturated glycerides. Similarly, although a somewhat less certain assumption is here involved, the polyethenoid (linoleic and linolenic) acids may be considered *vis-a-vis* oleic and saturated acids. These data, which are summarized in Table III, lead to the statements of the possible glycerides in each fraction A to F, and consequently in the whole fat, which appear at the foot of Table III.

The values for the component acids of the whole oil, obtained by summation from fractions A to F, agree with those obtained by the direct component acid analysis (Table II) within ±1.4 units per cent for linoleic and linolenic acids but differ by about ±2.5% for oleic and the total saturated acids. As already mentioned, the differences are probably due to cumulative analytical errors in the analysis of the six glyceride fractions.

The total proportions of tri-unsaturated (42%) and mono-saturated di-unsaturated (58%) glycerides indicated (Table III) in this specimen of soya bean oil are probably very close to the true values. The individual types (e.g., saturated dilinoleins, oleodilinoleins, oleolinoleolinolenins, etc.) have been calculated on the assumption that "even distribution" persists within the broader categories of tri-unsaturated and di-unsaturated triglycerides to the same degree that, as the experimental data have demonstrated, it operates in the production of these broader categories as a whole. The precision to be attached to the final detailed figures is, therefore, not equal to that for the tri-unsaturated and di-unsaturated glyceride groups as a whole, but it is almost certainly sufficient to give a fairly close estimate of the proportions of the glycerides which contain respectively one, two, or three polyethenoid (linoleic or linolenic) groups. This is the important factor from the point of view of a good "drying" oil, and the experimental data establish, for instance, quite unequivocably that soya bean oil does not contain more than about 5% of glycerides in which three polyethenoid groups (a linolenic with two linoleic radicals) are present.

In view of the technical processes which are coming into use for separating a concentrate of the more unsaturated part of soya bean oil as a fraction with improved "drying" qualities to those of the whole oil, it is of interest to note that the fractions C to F (soluble in acetone at or below —40°) obtained in our present investigation (Table II) are fairly sharply differentiated in iodine value from the more saturated fractions A and B. Fractions C-F formed 37% of the whole oil and had an average iodine value of 152. Published records (3, 4) of separations of this kind

TABLE III.

Component Glycerides of Soya Bean Oil.

	A	B	C	D	E	F	Whole oil
Glycerides % (mol.)	36.0	27.0	18.0	10.3	5.1	3.6	100.0
Component acids							
(Increments % mol.)							
Saturated	9.3	5.9	2.2	1.1	0.4	0.3	19.2
Oleic	9.7	5.2	3.5	2.0	0.8	0.6	21.8
Linoleic	14.7	13.5	10.2	5.8	3.0	2.1	49.3
Linolenic	2.3	2.4	2.1	1.4	0.9	0.6	9.7
Component glyceride groups							
(Increments % mol.)							
(a) Di-unsaturated mono-saturated	27.9	17.7	6.6	3.3	1.2	0.9	57.6
Tri-unsaturated	8.1	9.3	11.4	7.0	3.9	2.7	42.4
(b) Mono-polyethenoid	21.0	6.3	.....	.....	.....	.....	27.3
Di-polyethenoid	15.0	20.7	17.1	9.3	3.6	2.5	68.2
Tri-polyethenoid	.....	.....	0.9	1.0	1.5	1.1	4.5
Possible component glycerides							
(a) Saturated oleo-linoleins	18.3	5.4	.....	.....	.....	.....	23.7
Saturated dilinoleins	6.9	11.4	6.6	3.3	1.2	0.9	30.3
Saturated oleo-linolenins	2.7	0.9	.....	.....	.....	.....	3.6
(b) Oleodilinoleins	3.9	3.0	5.1	2.8	1.2	0.9	16.9
Oleo-linoleo-linolenins	4.2	6.3	5.4	3.3	1.2	0.6	21.0
Dilinoleo-linolenins	.....	.....	0.9	0.9	1.5	1.2	4.5

have hitherto not dealt with the detailed composition of the two fractions into which the soya bean oil was resolved, the criteria being confined to iodine values. It is, therefore, useful to illustrate from our present data the fatty acid composition and the chief glyceride types in those fractions of the present oil which were respectively *soluble* in acetone at —40° (or below) and *insoluble* in acetone at —30° and —40°. The relevant figures are given in Table IV.

TABLE IV.

Component Acids and Glycerides of Soya Bean Oil and of Its Two Main Fractions

	Whole soya bean oil	Fractions from acetone at —40°	
		Insoluble	Soluble
Glycerides (% mol.)	100	63	37
Iodine value	132.6	121.8	152.0
Component acids	% (mol.)	% (mol.)	% (mol.)
Saturated	19.2	24.2	10.7
Oleic	21.8	23.6	18.5
Linoleic	49.3	44.7	57.1
Linolenic	9.7	7.5	13.7
Component glycerides			
Di-unsaturated mono-saturated	58	73	32
Tri-unsaturated	42	27	68
Glycerides containing			
1 Saturated acyl group	58	73	32
2 Polyethenoid acyl groups	68	56	88
3 Polyethenoid acyl groups	5	....	12
1 Linolenic group	29	22	41

Fundamentally, the properties of a good paint oil of the linseed oil type would be expected to depend on the proportion of glycerides in which at least two and, better, all three acyl groups were those of linoleic or linolenic acids; whilst, *prima facie*, its quick "drying" qualities would be expected to be greater, the greater the content of linolenic acid in the mixed fatty acids of the oil. Employing these criteria, it is evident that the 30-35% or so of soluble fraction from soya bean oil should be definitely superior as a paint oil to the whole soya bean oil. It consists entirely of glycerides containing 2 or 3 polyethenoid groups (although the latter still form little more than 10% of the whole fraction), and its linolenic acid content is increased by about 50%, compared with that of the whole oil. (The portion of the oil insoluble in acetone at —30° and —40° (63% of the original oil) still contains about 7% of linolenic acid, equivalent to about 22% of glycerides in which this acid is present. Except for this it is similar in general fatty acid composition to a cottonseed oil and should be usable for edible fats, especially if hydrogenated sufficiently to convert all the linolenic and some of the linoleic glycerides to oleic, *iso*-oleic, and *iso*-linoleic glycerides).

Whilst the figures in Table IV illustrate the improvement to be expected in "drying" qualities of the 30-35% "soluble fraction" from soya bean oil, it is nevertheless important to bear in mind that, relative to linseed oil and to some other "drying" oils, this product would appear still to be considerably deficient in "drying" power. This will be readily appreciated from Table V in which we have collected the relevant data (as far as at present possible) for the various oils in question. The values for linseed oil are based on the work of Walker and Mills (7), and for lumbang (candlenut) oil (8) and rubber seed oil (6, 9) on data from our Liverpool laboratory. It will be seen that, judged either by linolenic acid content or by the proportion of glycerides containing either 2 or 3 polyethenoid (linoleic or linolenic) groups, the "soluble" soya bean oil fraction compares unfavourably not only with linseed oil but with either of the other two oils (each of which could doubtless be improved equally well by the low-temperature solvent process). It will be appreciated that this comparison is based solely upon the relative merits of the various fatty oils as indicated by their component acid and glyceride contents and does not include any consideration of the current relative availability of any of them.

TABLE V.

Comparison of the "Soluble" Fraction of Soya Bean Oil With Linseed and Other Drying Oils.

Oil	Linseed	Lumbang (Candlenut)	Rubber seed	Soya bean soluble fraction
	% (mol.)	% (mol.)	% (mol.)	% (mol.)
Component acids				
Saturated	10-15	13	21	11
Oleic	20-15	10	20	18
Linoleic	20-15	49	38	57
Linolenic	50-55	28	21	14
Glycerides containing				
1 Linolenic group	80-90 (?)	85	62	41
2 Polyethenoid groups	25-30	52	76	88
3 Polyethenoid groups	Ca. 65	33	0	12
i.e. 2 (or 3) Polyethenoid groups	Ca. 90-95	85	76	100

Summary

The mixed glycerides of soya bean oil consist of nearly 60% of mono-saturated di-unsaturated glycerides and about 40% of tri-unsaturated glycerides; two polyethenoid groups (chiefly linoleic) are present in

nearly 70% of the tri-glycerides, but less than 5% contains three in the same triglyceride molecule whilst linolenic groups occur (singly) in not much more than 25% of the soya bean glycerides.

Of the soya bean oil glycerides (37%) which remain in solution in acetone at —40°, about 90% contain two polyethenoid groups and about 10% contain all three acid radicals in this form whilst linolenic acid is present (singly) in about 40% of the glycerides; but over 30% of this fraction still contains one saturated acyl group.

Such a solvent-separated soya bean oil fraction would thus appear to be considerably less efficient as a drying oil than certain whole oils such as those of rubber seed or candlenut (lumbang), the latter in turn not possessing so great a linolenic acid content and content of linoleo-linoleno-(tri-unsaturated) glycerides as linseed oil itself.

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A Laboratory Method for Determining the Ability of Antioxidants to Stabilize Fat In Baked Goods[1]

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A LARGE volume of work has been published on the use of a wide variety of compounds to stabilize fats against oxidative rancidity. To a large extent the only property investigated has been the ability of the individual compounds to retard the rate of deterioration of the fat itself, as measured by various organoleptic and chemical tests. Rather recently more attention has been directed toward the development of practical antioxidants which possess such essential characteristics as nontoxicity, stability, lack of odor or flavor, fat-solubility and the ability to stabilize the fat in baked goods (1).

The literature contains relatively few references to the ability of antioxidants to carry their stabilizing effect over into the fat in bakery products. Higgins and Black (1) reported that essentially all of the stabilizing effectiveness of gum guaiac and a large portion of that of nordihydroguairetic acid (N.D.G.A.) were carried through to crackers. Lundberg, *et al.* (2), explained this partial failure of N.D.G.A. on the basis of its instability to the alkalinity of crackers. These authors found the carry-over of N.D.G.A. to be better in pie crusts, which are not alkaline, than in crackers. Tocopherols and propyl gallate have shown very little tendency to stabilize crackers (1).

The reasons for such variations were described in 1936 in a patent by Richardson, Grettie, and Newton (3), who discovered that substituted polyphenols and polyphenol derivatives which were soluble in oil or fat and relatively insoluble in water were effective not only in stabilizing the oil or fat as such but also in retarding the oxidation of the fat after it had been used as shortening in bakery products. Phenols which were relatively soluble in water were said to be extracted from the fat when the shortening is mixed with other ingredients containing moisture; this was especially true if the other ingredients were alkaline in reaction. To our knowledge, no further reference has been made in the literature to this broad concept.

One of the reasons for the scarcity of such publications has undoubtedly been the fact that many laboratories do not have the facilities for testing fats in baked goods. For that reason it was felt that it would be of value to develop a laboratory method for assaying the ability of an antioxidant to stabilize such products. The above solubility concept appeared to offer the most promising basis for such a test, and an investigation was undertaken in that direction. It has been found that the relative partition of an antioxidant between a fat and hot water can be correlated with its ability to stabilize baked goods; those antioxidants which are found almost entirely in the water phase are practically ineffective while those which are not extracted by water but remain in the fat are effective in retarding rancidity in various bakery products.

Experimental

General Method of Partition Analysis. A standard method was used in all the partition analyses. It will be evident in the subsequent discussion that many variations of the method may well be employed; for reliable comparison, however, it is felt that the same partition method should be used throughout any given series of tests. In the present work sufficient antioxidant was added to lard to represent 0.05% concentration, assuming complete solubility. Although the lard was then held at 170° F. for about 2 hours, some of the compounds were not completely dissolved. In all cases the lard was filtered to remove undissolved material.

To a 100-gm. sample of the solution of antioxidant in lard was added 100 gm. of distilled water. The fat-water mixture was heated to the boiling point of water and then held on a steam bath for 15 minutes with intermittent stirring. After being transferred

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