TABLE III

Melting Point Determinations on Prepared 2,4-Dinitrophenylhydrazones of 7-, 10-, and 11-Carbon *N* Aldehydes and Compounds Comprising Bands 1 and 2

	Determined Melting Point (corr.)	Reported Values
	°C.	°C.
n-Heptanal DNPH	106.3	106.0 (3)
n-Decanal DNPH	103.5	104.0 (3)
n-Undecanal DNPH	104.0	104.0 (3)
Band 1 DNPH	87.0	......
Mixed Decanal (25%) and Undecanal (75%) DNPHs	84.0	......
Mixed Heptanal (50%) and Undecanal (50%) DNPHs	75.0	......
Mixed Band 1 and Undecanal (approx. 50%:50%) DNPHs	81.0	......
Band 2 DNPH	97.0–97.5	......
2-Decenal DNPH	......	128.8 (4)
2-Undecenal DNPH	......	122.6 (4)

stable states, as discussed by Braddock *et al.* (6) must also be reckoned with here. However, apart from melting point variations and transition points, discussed in some detail by Wahhab (7), we have never observed changes in absorption spectra of our derivatives after crystallization by different methods and subsequent dissolution. Stadtman's spectrum of furfural DNPH (8) is readily reproducible and would be described as the *trans*-form as judged by a transmittance curve given by Braddock *et al.* (6). This is clearly the stable form and the only one repeatedly encountered on the chromatogram under our conditions. It is unlikely therefore that the number of components isolated in this work will be greatly reduced by duplication as metastable forms. However the method obviously may be of great utility, as Kawahara, Dutton, and Cowan (9) have shown, in isolating fractions from a single source such as methyl linolenate.

Summary

Fourteen petroleum ether-soluble and 16 insoluble DNPH derivatives have been isolated from rancid corn oil and their properties discussed. Evidence suggests that n-undecanal and n-undecenal are among the components of the rancid oil.

TABLE IV

Extinction Coefficients of Prepared 2,4-Dinitrophenylhydrazones and of Unknown Bands 1 and 2

	Heptanal DNPH	Decanal DNPH	Undecanal DNPH	Band 1 DNPH
Specific Ext. Coeff.:				
357 mμ	70.30	66.90	58.10	58.85
250 mμ	38.90	34.15	30.30	32.05
225 mμ	50.10	46.25	40.10	43.40
Molecular Weight of DNPH	294	336	350	347[a]
Molecular Ext. Coeff.:				
357 mμ	20,700	22,490	20,340	20,450
250 mμ	11,440	11,470	10,610	11,130
225 mμ	14,740	15,560	14,050	15,080

	Band 2 DNPH			2-Decenal DNPH (38)	2-Undecenal DNPH (4)
Specific Ext. Coeff.:					
376 mμ	78.5	78.5	78.5	86.3	81.7
247 mμ	47.3	......	......	......	......
Molecular Weight of DNPH	343[a]	334[b]	348[c]	334	348
Molecular Ext. Coeff.:					
376 mμ	26,930	26,250	27,360	28,850	28,440

[a] Assumed on basis of *N* content.
[b] Assumed on basis of 2-decenal.
[c] Assumed on basis of 2-undecenal.

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Solubility of Monoglycerides in Oil and Its Relation to the Production of Global Edible Spread[1]

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This paper reports the solubility of monoglycerides in vegetable oil and the influence of tempering monoglyceride suspensions on solubility behavior. Plastic mixtures of saturated monoglycerides and vegetable oil exhibit uniform consistency over a wide range of temperature and, as formulated for edible use, have been proposd as a global spread suitable for use in extremes of temperature. Provided it has been adequately tempered after solidification in manufacture (9), the spread leaves the mouth without a tallowy sensation even though it does not melt at body temperature. Physical studies have been made to reveal the basis for changes caused by tempering, and in a previous report (5) it was established by X-ray diffraction and microscopic observations that monoglycerides in spreads recrystallized during tempering. The low solubility of monoglycerides found in the present investigation explains the small temperature dependence of global spread consistency between 32° and 100°F. (0°-38°C.). Solubility data also confirm that the monoglyceride precipitated by rapid chilling in the production of global spread is present in an unstable state, which is stabilized by tempering.

Experimental

Materials. To reveal the influence of fatty acid composition on solubility, samples of essentially pure stearic and palmitic acid monoglycerides were investigated. Four molecularly distilled monoglycerides were prepared by Distillation Products Inc.,[2] from the following materials: a) commercial high-purity stearic acid, b) commercial palmitic acid more than 90% pure, c) triple-pressed stearic acid containing

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[1] Presented at the fall meeting of the American Oil Chemists' Society, Minneapolis, Minn., Oct. 11-13, 1954.

approximately 45% stearic and 55% palmitic acids, and d) completely hydrogenated lard containing approximately 70% stearic and 30% palmitic acids. Periodate oxidation analyses at this laboratory indicated monoglyceride purities to be 98.3, 91.0, 95.8, and 97.4% of the 1-monomer, respectively. In order better to correlate solubilities with the properties of global spreads, these commercial monoglycerides were used without further purification.

All solubility measurements were made in commercial cottonseed salad oil as the liquid phase.

Methods. Weighed amounts of monoglyceride were dissolved in known weights of cottonseed oil, and approximately 10-ml. aliquots were placed in test tubes for observation of temperature of complete dissolution (clear points) and temperature of initiation of precipitation (precipitation points). Solutions containing from 0.1 to 20.0% monoglyceride (gram per 100-g. total) were cooled slowly in a water bath from 77° to 28°C. Solidified samples for clear point tests were held 24 hrs. at 4°C. and then tempered 5 days at 28°C. Clear points were noted as the bath temperature was increased from 28° at ¼°C. per minute. After determining clear points, all samples were heated to 77° and cooled approximately 2°C. per minute for the determination of precipitation points. Solubility data on samples thus prepared appear in Table I. During the experiments, tube contents were stirred by a brass rod terminating in a 1.0-cm. loop and driven by a reciprocating vertical motion. At the above rate of heating there was less than 0.1°C. difference between bath and sample temperatures. At the selected rate of cooling a 2-5°C. difference existed between bath and sample temperatures, and for this reason precipitation points were read on a thermometer immersed in oil. The rate of cooling was a practical intermediate between fast and slow chilling and was equivalent to cooling rates used by other workers for thermal analysis (8, 11, 12). Temperatures were read on a National Bureau of Standards calibrated thermometer, graduated in 0.2° C. intervals.

TABLE I

Solubility of Monoglycerides in Cottonseed Oil

Concentration[a]	Transition points of monoglycerides prepared from:							
	Stearic acid		Hydrogenated lard		Triple-pressed stearic acid		Palmitic acid	
%	clear °C.	ppt. °C.	clear °C.	ppt. °C.	clear °C.	ppt. °C.	clear °C.	ppt. °C.
0.1	35.7	......	32.5	......	......	......	......	......
.25	40.5	12.5	38.0	12.8	43.0	......	37.0	......
.50	45.3	22.0	44.0	20.0	44.5	16.0	42.0	13.5
1.00	51.6	32.0	49.6	32.8	48.5	26.5	46.8	24.0
2.50	59.8	44.0	56.0	41.5	55.0	38.5	54.7	36.0
5.00	66.0	52.5	61.7	48.9	61.0	46.5	61.3	45.2
10.0	69.6	58.2	65.0	55.8	65.0	53.0	64.7	51.3
20.0	72.0	65.3	67.8	60.0	66.5	55.0	66.8	58.0

[a] gram/100 gram total weight.

Effects of quick chilling and tempering at temperatures equal to those which occur in global spread production (9) were also investigated. For this purpose two sets of samples were brought to 77°C. and, while stirred, were rapidly chilled in circulating water at 12°C. Samples containing less than 0.2% monoglyceride were further chilled at 4°C. Clear-point determinations on one set of quickly chilled samples were made with the water-bath temperature set within a degree of the anticipated clear point so that tempering during the determination would be minimized. The other set of quickly chilled samples was tempered 18 hrs. at 45°, cooled to 25°C., and then used for clear-point determinations as described earlier. Samples containing less than 1% monoglyceride dissolved completely at 45°C. and hence were tempered instead at 25°C. for 7 days. The solubility data for tempered and untempered samples appear in Figure 1.

X-ray diffraction patterns of the crystalline solids, from which excess oil had been absorbed by filter paper, were made with filtered CuK$_\alpha$ radiation and a Geiger-Muller diffractometer.

Results and Discussion

Dissolution and Precipitation of Monoglycerides. Table I shows the temperatures at which monoglyceride suspensions at various concentrations in cottonseed oil cleared on heating, and at which the first precipitate formed on cooling homogeneous solutions. In the concentration range 0.25-10% monoglyceride, solubility increased approximately fourfold for each 10°C. rise in temperature. This temperature coefficient of solubility is half as large as that reported for tristearin in triolein or for hydrogenated cotttonseed oil (I.V. = 1.1) in cottonseed oil (13). Preparations containing 10-20% monoglyceride, which is the concentration range useful for plastic spreads, clarified near 70°C. for monostearin and about 65°C. for spreads containing monopalmitin. Clear-point solubility of the monoglycerides listed in Table I, irrespective of the relative amounts of monopalmitin and monostearin, was approximately 0.25% at body temperature (37.5°C.). Uniform consistency in the interval 32°-100°F. (0°-38°C.) for plastic spreads formulated with monoglyceride may be attributed to low solubility of the solid component. It can be noted that monopalmitin was more soluble than monostearin and that the solubility increased as the proportion of monopalmitin in the mixture of monoglycerides increased.

Monoglyceride first precipitated from solutions cooled at a moderate rate (2°C. per minute) when the monoglyceride concentration was 5 to 10 times greater than apparent saturation concentrations obtained by clear points of equivalent samples. The supercooling necessary to cause precipitation of monoglycerides, as discussed subsequently, may be associated with differences in the nature of the precipitated solid from that of the solid present in clear-point determinations.

Influence of Crystal State on Solubility. Figure 1 shows the temperature of complete dissolution for quickly chilled and tempered mixture of hydrogenated lard monoglyceride in cottonseed oil. It can be noted that tempering decreased solubility from 20% to 50%, depending upon concentration. Clear-point temperatures of samples chilled quickly and tempered at 45° and 25°C. (Figure 1) are probably stable limits since they lie on the same smooth curve as samples solidified slowly and tempered 5 days at 28°C. The 0.25% and 0.50% points of Table I which involved tempering at 28°C. are plotted on the tempered line of Figure 1.

Decreased monoglyceride solubility observed to accompany tempering might result from an increased

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[2] The mention of firm names or trade products does not imply that they are endorsed or recommended by the U. S. Department of Agriculture over other firms or similar products not mentioned.

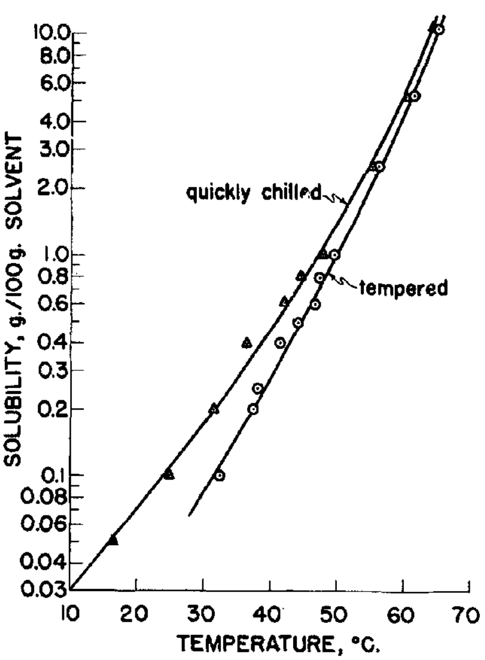

FIG. 1. Effect of tempering on clear-point solubility of monoglycerides from hydrogenated lard in cottonseed oil. Concentrations of 0.25% and 0.50% were taken from Table I and were tempered at 28°C. Other points below 1% were tempered at 25°C.; above 1%, at 45°C.

particle size. Present evidence indicates however that alteration in particle size causes marked changes in solubility only in the size range 10-100Å (4). Microscopic examination of the suspensions used for solubility determinations showed that tempering may have caused a slight increase in size; however monoglyceride particle size in both tempered and untempered systems was dominantly in the range 1-5 μ, which is at least 100 times greater than the size range critical for solubility. Solubility changes such as those found by Hulett (7) for sulfates and fluorides in the range 0.1-2 μ are now considered (4) to arise from lattice defects resulting from grinding to reduce particle size.

Rapid chilling in our experiments and the concomitant rapid precipitation might cause crystal lattice defects in the monoglycerides. To investigate this aspect, X-ray diffraction patterns of the monoglyceride were obtained from rapidly chilled suspensions and from similar suspensions after subsequent tempering for 18 hours at 35°C. Figure 2 presents the diffractometer tracings from the solids of 10% monoglyceride suspensions. The prominent side spacing of 4.55Å for both samples indicated that the β-crystal form occurred in both samples. Failure to observe diffraction maximum in the range from 20.5°-22° 2Θ corresponding to an interplanar spacing in the range d = 4.30Å-4.00Å, in the pattern of the untempered sample indicated absence of the sub-α, α, and β′ polymorphic forms. The most prominent difference between the patterns was intensification of diffraction maxima in the tempered sample, from planes of both long and short spacings.

Judged from the small area under the diffraction maxima in comparison to the large area of the background, it was concluded that only a small amount of the solids in the untempered sample was in an ordered or a crystalline state. The remainder of the sample possessed, at most, a low degree of order. Tempering increased the area of diffraction maxima, decreased the background area, and thus was concluded to have increased the proportion of crystalline material. It is unlikely that changes in particle size caused the observed changes in background and line intensity. X-ray diffraction line width and intensity changes occur only for particle sizes less than 10^{-5} cm. (2), which are at least $\frac{1}{10}$ of the size microscopically found for most of the particles in the suspensions. On the basis of the X-ray diffraction results therefore it was concluded that decreased solubility of tempered samples results from better molecular order of the tempered solid monoglyceride.

The discrepancy in the apparent equilibria for precipitation and dissolution discussed in the previous section, and shown in Table I, may arise from the condition that precipitation point determination involved equilibration of oil phase with imperfectly organized solid whereas the clear-point determination involved equilibration of the oil phase with tempered and more ordered crystalline material.

The disordered state of the rapidly chilled monoglyceride precipitated from oils may be similar to the "vitreous state" described by Malkin (12) in his reports on rapid chilling of triglycerides from melts, or to a "low extent of stabilization" in the terminology of Lutton (10). In our experiments rapid chilling of oil solution produced a disordered variant of the β-polymorphic form normally obtained on slow precipitation of monoglycerides from solvents, much as Malkin obtained the vitreous state by rapid chilling of a melt and considered it to be a disordered variant of the α-form. In a discussion of polymorphic forms of monoglycerides crystallized from melts (12) Malkin has remarked on slowness of crystallization and crystalline transformations.

We have reported previously that the solids content of freshly prepared global spread stored at low tem-

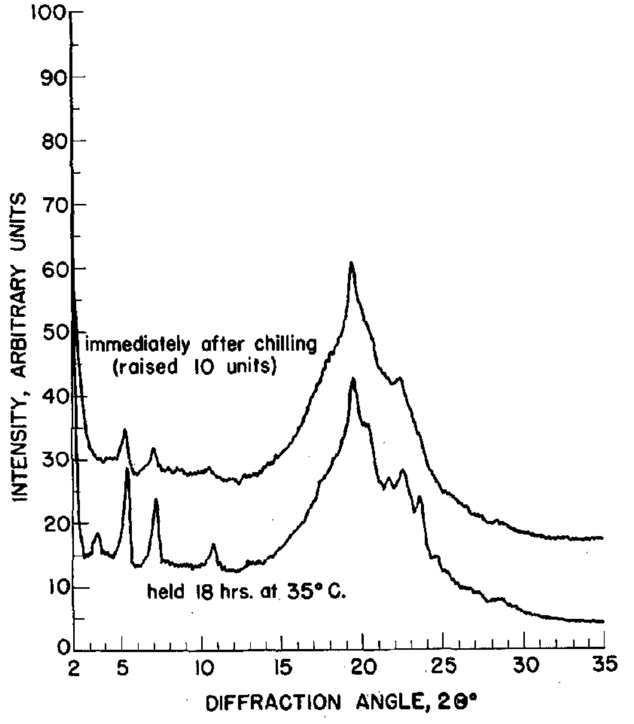

FIG. 2. Effect of tempering on the X-ray diffraction spectrum of monoglycerides from hydrogenated lard which had been quickly precipitated in cottonseed oil.

peratures was slightly lower than that found after tempering (5). Once extensive precipitation of solids has occurred, it is unlikely that appreciable supersaturation would persist in the spread since the system then is seeded heavily. To account for the lower solids content, the solid obtained by rapid chilling must be presumed to be more soluble. This has been confirmed by solubility measurements. The 0.5-1.5% total weight increase in solids content reported previously to result from tempering global spread is somewhat higher than the difference in solubility at 30°C. caused by tempering as exhibited in Figure 1. The solubility data at that temperature however was obtained with precipitates from dilute solutions and hence may not be applicable quantitatively to precipitated systems with higher concentration of monoglycerides. The increase in solids content on tempering monoglyceride spreads is in agreement with the observations on cocoa butter by Jensen (8) and on artificial "hard butter" by Bailey (1) but is the reverse of findings on hydrogenated oils by Fulton (3) and on beef fat by Hofgaard (1, 6). Perhaps increased knowledge of oil solubility of triglycerides in various polymorphic forms or crystal states would clarify these apparent discrepancies. As previously reported (5), the paradox remains that tempering increases solids content and simultaneously decreases consistency of global spreads.

X-ray diffraction and microscopic observations reported here on dilute monoglyceride systems used for solubility determination confirm previous observations of crystal changes in spreads. In both dilute and concentrated systems, crystal edges became more sharply defined during tempering, and, in addition, an increase was noted in particle size. It therefore appears that conversion of a compact precipitate, containing poorly crystalline monoglyceride, into more perfect, larger, discrete crystals should be considered the dominant factor in causing softening on tempering and may also impart flow properties which contribute to better mouthing qualities.

Summary

Solubility data have been presented for monostearin, monopalmitin, and monoglycerides of triple-pressed stearic acid and hydrogenated lard in cottonseed oil. Monoglyceride precipitated from solution by quick chilling became less soluble after tempering 18 hrs. at 45°C. Decreased solubility was related to increased crystallinity as determined by X-ray diffraction. Solubility data thus confirmed conclusions previously reported that tempering operates to improve global spread primarily through recrystallization which is now shown to involve converting precipitates of low degree of molecular order into more perfect crystals.

Acknowledgment

The authors are indebted to E. P. Jones for the monoglyceride analyses.

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Component Fatty Acids in Body Fats of Some Marine Fishes

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THE FATTY ACID composition and complex nature of depot and body fats of many species of fish have been investigated. Though Lovern and his coworkers (1) have paid particular attention to depot fats, and the body fats of some fresh-water fish have been studied by Pathak and his collaborators (2), less attention seems to have been given to the composition of the body fats of marine species. The purpose of the investigations now reported was to study the composition of three species of marine fish caught along the coast near Bombay. These species were: white pomfret (*Stromateus cinerus*); black pomfret (*Stromateus niger*); and pala (*Hilsa ilsha*).

Samples and Methods of Analyses

Muscles from the freshly caught fish were autoclaved at 15 lbs. pressure at 250°F. for 15 min. The lipides were then extracted with diethyl ether, obtaining about 100 g. of oil representing the body fat of each species. Some characteristics of the oils are given in Table I.

The oils were saponified, and the mixed fatty acids were prepared for analysis by the procedure of Hilditch (3). The acids were fractionated by low temperature crystallization in diethyl ether and acetone (10 ml./1 g.) to obtain fractions having different iodine values.

The fractions were methylated, and the methyl esters in each were fractionated by low pressure distillation (4). The yields, iodine values, and saponification equivalents of each fraction of the methyl esters were determined.

TABLE I

Some Characteristics of Body Fats of Marine Fish

Fish	Oil		Mixed Fatty Acids	
	Iodine Value (Wijs)	Saponification Equivalent	Iodine Value (Wijs)	Saponification Equivalent
White pomfret	78.3	276.1	81.1	272.5
Black pomfret	105.3	283.7	113.7	278.8
Pala	77.4	277.4	81.4	271.0