TABLE I

Recovery of Free Carbonyl Compounds after Regeneration from Their DNP-Hydrazones

Carbonyl compound carbon chain length	Mg. for regeneration	Mg. recovered	Percentage recovery
n alkanals			
n-C5	1.05	0.438	42
n-C10	0.681	0.333	49
n-C12	1.35	0.669	50
n-alkan-2-ones			
n-C5	0.997	0.328	33
n-C9	1.61	0.665	41
n-C11	0.82	0.502	61
n-2-alkenals			
n-C5	0.446	0.257	57
n-C7	0.475	0.257	54
n-C8	0.744	0.413	56
n-C9	1.27	0.538	42
n-C10	0.984	0.306	31
n-C11	1.11	0.252	23

Recoveries for a number of alkanals, alkan-2-ones, and 2-alkenals are presented in Table I. Aside from these compounds, a number of miscellaneous carbonyl compounds have been regenerated and their odors found to be typical of the authentic compounds. These included 3-methylthiopropanal, furfural, phenylacetaldehyde, cinnamaldehyde, and the bis-DNP-hydrazone of 2,3-butanedione.

Discussion

Low recoveries shown in Table I may be attributed in part, to the manipulation required to obtain the quantitative data. Part of the loss was caused by steam distillation because isolation by this procedure varies among compounds. The extent of reaction of the distilled carbonyl compounds with DNP-hydrazine reagent may be limited at the concentration used here (2). Consequently it is not known whether the procedure will regenerate each carbonyl compound equally well.

With the alkanals and alkan-2-ones, there was no evidence that the regeneration methods caused the production of additional carbonyl compounds. On regeneration of 2-alkenals however traces of additional carbonyl compounds were produced. The trace components appeared as faint bands on the chromatography column that were not identified. One compound moved before the original "enal," and two very small bands followed it. In every recovery the bulk of the carbonyl compounds regenerated from the DNP-hydrazone of the 2-alkenal was the original "enal."

The advantage of the sulfuric-acid regeneration method over other methods is that it is fast and simple, and the odor of the regenerated carbonyl is easily observed. This procedure has been particularly useful in flavor chemistry research and in studying oxidation products of lipids.

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The Preparation and Purification of Monoglycerides.

I. Glycerolysis of Oils[1]

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Mono- and diglycerides were produced by reacting the following oils with glycerol: coconut, peanut, sesame, linseed, and sardine. It was shown that the yield of monoglyceride was not dependent upon the fatty acid composition of the oil but on the solubility of glycerol in oil, which is dependent in part on the temperature. An excess of glycerol above that which is soluble does not change the composition of the reaction product. At 180°C. no more than 45% monoglycerides can be formed by glycerolysis.

MONOGLYCERIDES are important commercial products used for various purposes. The two important processes used in industry for preparing monoglycerides are the direct esterification of fatty acids and glycerol and the glycerolysis of oils, *i.e.*, the reaction of an oil with glycerol.

Of the two methods, the glycerolysis method has become more important because the fatty acid does not have to be liberated from the fat. The method consists in heating the glycerol with fats at 180–250°C. in the presence of catalysts such as caustic soda or sodium alcoholates in amounts of 0.05 to 0.20% of the weight of fat used. Along with monoglycerides, di- and triglycerides are also produced. The reaction is conducted at a high temperature to hasten the rate of reaction and to increase the miscibility of the reaction mixture with glycerol. The extent to which the reaction can be carried out is limited by the comparative immiscibility of glycerides and glycerol. Any glycerol forming a second liquid phase cannot participate in the reaction.

The maximum amount of glycerol miscible and reactable with highly hydrogenated cottonseed oil plus 0.1% NaOH was determined by Feuge and Bailey (1). The solubilities of other oils such as coconut, linseed, and sesame in glycerol were determined by Choudhury (2). It was concluded from this work that the solubility of oil in glycerol was mainly dependent on temperature, and slightly dependent on molecular weight and the unsaturation of the oil.

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There are many articles in the literature which purport to show that factors such as temperature, the presence of catalysts, and fat composition affect the composition of the reaction mixtures of glycerol and fat. Feuge, Bailey, and Kraemer (3) have shown that glycerolysis in an homogeneous medium proceeds substantially according to chance, producing at equilibrium a reaction mixture in which the fatty acid radicals are distributed and esterified at random among all available hydroxyl groups. According to them, the composition of the product in terms of mono-, di-, and triglycerides can be calculated from the proportions of the reactants, provided that the final mixture is homogeneous.

The temperature which is usually employed in glycerolysis reaction is 175–250°C. Higher temperatures cannot be used because they cause both the glycerol and the oil to polymerize. The maximum amount of glycerol miscible with oils at 175–200°C. is, according to Choudhury (2), about 20 to 22% on the basis of the weight of the fat. According to Feuge *et al.* (3), the composition of the glycerides will be fixed regardless of the amount of excess glycerol that is used. There have been many papers recently stating that by glycerolysis reaction it is possible to obtain much higher amounts of monoglycerides (4,5,6).

Mehta *et al.* (7) have carried out glycerolysis of peanut and mustard oil in the presence of catalysts such as lime and litharge. According to them, the nature of the component acids present in the oil influences the relative amounts of mono- and diglycerides formed.

The data existing on the effect of the molecular weight and the unsaturation of fatty acids on the ultimate equilibrium concentration of monoglyceride in the fatty product at a particular temperature are far from complete.

The purpose of the present investigation was to study the glycerolysis reaction in order to answer the following questions:

1. how the reaction actually proceeds at temperature where glycerol decomposition is completely suppressed;
2. whether the mol. wt. or the unsaturation of the fat has any influence on the proportions of mono-, di-, and triglycerides formed at different intervals up to the equilibrium stage in the glycerolysis process.

Experimental

The oils selected for the study along with their properties are shown in Table I. Each of the oils was refined according to the standard A.O.C.S. procedure. The glycerolysis reaction were carried out in a 3-necked round-bottomed flask immersed in a constant temperature bath at 180°C. The flask was provided with a high-speed glass stirrer passing through a stuffing box in the central neck. Nitrogen was allowed to enter the reaction vessel in measured amounts. A slight suction was maintained to carry away the water formed in the reaction but not to carry away the glycerol.

All the experiments were carried out with lots of 100 gms. of oil. The weighed amount of glycerol was poured into the reaction flask, and the catalyst (0.1 gm. NaOH) was added to it. When the reaction temperature was attained, the weighed amount of oil preheated to the same temperature was poured into the flask. Reactions were carried out with each of the oils and amounts of glycerol from 10 to 25%, at times from one to six hours. Samples were drawn quickly from time to time to study the rate and manner of the reaction.

TABLE I

Physico-Chemical Constants of the Oils

Oil	Iodine value	Hy-droxy value	Mean molecular weights				Glycerol from oil
			Fatty acids	Triglyc-erides	Diglyc-erides	Mono-glycer-ides	
							G. per 100 g.
Coconut	6.1	3.1	200.1	638.3	456.2	274.1	14.49
Peanut	94.9	4.5	279.8	877.3	624.2	360.1	10.50
Sesame	107.2	3.5	279.3	875.8	615.4	353.3	10.52
Linseed	181.7	2.7	278.3	872.9	602.6	352.3	10.52
Sardine	178.5	1.5	300.2	938.6	656.4	374.2	9.80

Treatment and Analysis of Reaction Product

The fatty product was taken into solution in ether, and the ether solution washed free of uncombined glycerol by washing with 20% solution of sodium sulphate in water. The ether solution was dried over anhydrous sodium sulphate, and the ether was evaporated under vacuum.

Alpha-monoglyceride content of the product was determined by the official A.O.C.S. method (8). The hydroxyl value was determined by the method of Hind

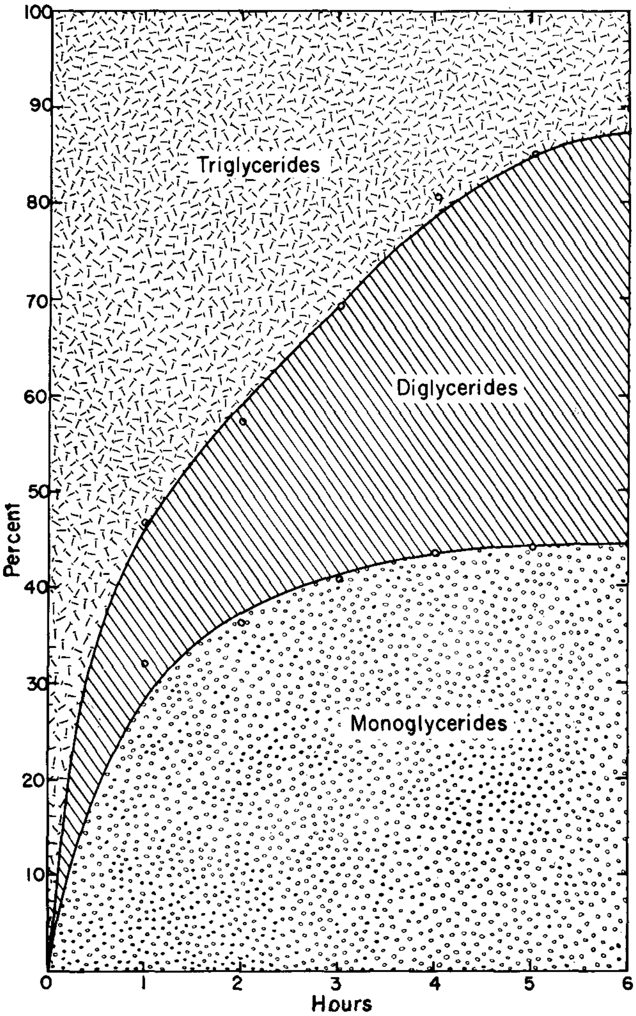

FIG. 1. Products of glycerolysis of linseed oil for various time intervals. 25% glycerol, 0.1% NaOH, temp. 180°C.

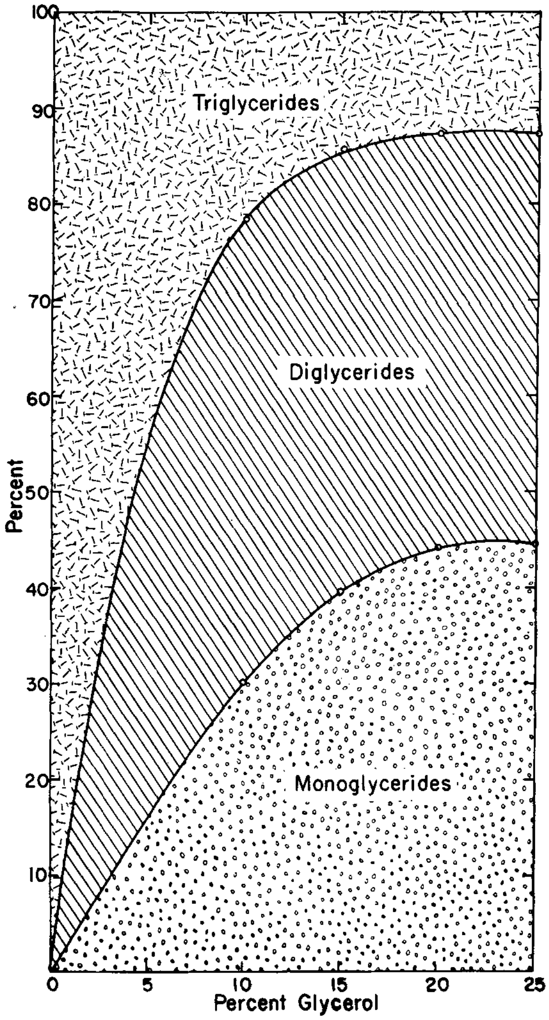

FIG. 2. Products of glycerolysis of linseed oil with different amounts of glycerol. Time 6 hrs, 0.1% NaOH, temp. 180°C.

et al. (9). From the monoglyceride content and the hydroxyl value, the composition of the reaction product was calculated.

Results

Typical data for the progress of the glycerolysis reaction with time are shown for linseed oil in Table II and Figure 1. Results of glycerolysis of different oils for six-hour periods with varying quantities of glycerol are given in Table III and results for linseed oil in Figure 2.

These data show that there always exists an equilibrium state where there is no appreciable change in the different percentages of the component glycerides. The results corroborate the findings of Feuge *et al* (3). The equilibrium stage is attained within four to five hours after starting the reaction in all cases studied.

TABLE II

Products of Glycerolysis of Linseed Oil with 25% Glycerol[a]

Time in hours	Composition of glycerides in product		
	Mono-	Di-	Tri-
	%	%	%
1	32.0	14.7	53.3
2	36.3	21.0	42.7
3	40.9	28.5	30.6
4	43.5	37.2	19.3
5	44.1	40.9	15.0
6	44.5	42.8	12.7

[a] 0.1% NaOH, 180°C.

TABLE III

Products of Glycerolysis of the Oils with Several Ratios of Glycerol for Six Hours[a]

Oil	Glycerol	Composition of glycerides in product		
		Mono-	Di-	Tri-
	%	%	%	%
Coconut	10	32.4	46.5	21.1
	15	41.8	43.7	14.5
	20	45.1	44.0	11.0
	25	45.2	44.1	10.7
Peanut	10	29.2	49.1	21.7
	15	39.1	46.3	14.6
	20	43.5	43.7	12.5
	25	44.1	43.2	12.7
Sesame	10	31.2	48.7	20.1
	15	39.9	47.1	13.0
	20	43.7	42.9	13.4
	25	44.2	43.3	12.5
Linseed	10	30.1	48.2	21.7
	15	39.6	46.1	14.3
	20	44.1	43.2	12.7
	25	44.5	42.8	12.7
Sardine	10	30.9	48.2	21.3
	15	39.5	47.1	13.4
	20	44.2	43.8	12.0
	25	44.5	43.1	12.4

[a] 0.1% NaOH, 180°C.

When a low amount of glycerol is reacted with the fat, the proportion of monoglyceride at equilibrium concentration is much less than the proportion of diglyceride in the reaction product. As the glycerol concentration is increased, this difference between mono- and diglyceride diminishes gradually; and ultimately when 20 and 25% glycerol are used, the proportion of mono- and diglycerides in the reaction product at the equilibrium stage is almost the same or the monoglyceride is slightly higher. The concentration of monoglyceride in any case does not exceed 45%.

The triglyceride percentages diminish with a constant rate up to the fourth hour of the reaction in almost all cases, after which the lowering of triglyceride concentration is comparatively slower and ultimately the concentration of triglyceride becomes constant.

As regards the effect of fatty acid composition on the ultimate percentages of mono-, di-, and triglycerides, it is found that there is no marked effect up to 20% glycerol concentration. As has been shown by Choudhury (2) the solubility of glycerol in oils at 100–200°C., is 20 to 22%. Under these conditions, all the glycerol is miscible with the oils and the number of hydroxyl groups available to the fatty radicals are the same for all the oils. The yield of monoglyceride is determined by the random distribution pattern of Bailey and co-workers (3). When the data obtained by the present investigator are compared with the yield of monoglyceride as predicted by Bailey, it is found that the experimental results are in fair agreement with the statistically predicted ones resulting from a random distribution pattern. It can be concluded, therefore, that the fatty acid composition of the fat does not have any effect on the ultimate percentages of mono-, di-, and triglycerides, so long as the glycerol is miscible with the oil.

When 25% glycerol was used, not as high a monoglyceride yield was obtained as reported by other workers. This is because at 180°C. the maximum amount of glycerol miscible is 20 to 22%. If we add an excess of glycerol, it simply forms a separate layer and does not react with the fat. No matter how much excess glycerol is used, there will be very little change in monoglyceride yield.

In the present work the presence of 2-monoglycerides in the reaction product has been neglected because it is generally assumed that the amount of 2-monoglycerides formed in this reaction is small. If the presence of 2-monoglycerides had been taken into account, it would have resulted in slightly more monoglyceride than shown by the standard method and less diglyceride than calculated.

Conclusions

The yield of monoglyceride is not dependent on the fatty acid composition of the oil but on the solubility of glycerol in oil, which is more or less dependent upon temperature.

No matter how much excess glycerol is used, the ultimate composition of the reaction product in a glycerolysis reaction is fixed; and this depends on the amount of glycerol miscible with the oil.

At 180°C. no more than 45% monoglyceride can be obtained by glycerolysis.

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A Kinetic Study of the Autoxidation of Methyl Linoleate and Linoleic Acid Emulsions in the Presence of Sodium Chloride[1, 2]

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Autoxidation of linoleic acid and methyl linoleate emulsions in aqueous buffer solutions was studied by the rate of oxygen uptake. The oxidation rates of methyl linoleate emulsions increased with an increase in the pH of the buffer solution. With linoleic acid, oxidation rates rose until the increase reached its peak at pH 5.50 and then decreased gradually to a minimum at pH 8.00.

Oxidation rates of methyl linoleate and linoleic acid emulsions decreased with increased concentration of NaCl in the system. The effect of variation of pH of the emulsion in the range investigated was similar to that in emulsions without NaCl. There was no evidence that NaCl accelerated the oxidation rates in the system.

The observed inhibitory effect of NaCl may result from the decreased solubility of oxygen in the emulsion with the increased concentration of NaCl. Consequently the availability of oxygen would be a limiting factor in oxidation rates.

The activation energy for the monomolecular and bimolecular reactions of methyl linoleate and linoleic acid autoxidation was found to be independent of the pH value and sodium chloride concentration of the system. The energy of activations for the monomolecular and bimolecular reactions of methyl linoleate and of linoleic acid are 22,000, 18,200, 19,600, and 16,400 cal./mol., respectively.

Spectrophotometric studies of the autoxidized emulsions of linoleic acid and its methyl ester indicate that the magnitude of the absorption at 2325Å is the same at different pH values. On the contrary, the secondary products showing absorption at 2775Å are to some extent dependent on the pH value of the emulsion.

DEVELOPMENT OF RANCIDITY in foods often involves an aqueous fat system. Comparatively little work has been done in such systems, and it is often difficult to draw any general conclusion as moisture may influence the solubility of accelerators or inhibitors. Lea (22), in reviewing the literature, reported that the fat in most powdered foods, crackers, and other cereal foods becomes more rancid as moisture content is lowered to extremely low values. On the contrary, moisture was found to have an accelerating effect on the oxidation of lard. Chang and Watts (7) observed that the effect of NaCl on fat oxidation depended greatly on the amount of moisture in the system. Later Spetsig (30) reported that methyl linoleate oxidized faster in the dry form than in the presence of water.

No common meat additive has a more profound or more puzzling effect on oxidative changes than sodium chloride. In 1934 Lea (20) reported that sodium chloride accelerated oxidation of pork fat. Later, in 1936, he (21) cited several studies in which NaCl solutions either had no effect or actually inhibited rancidity in various foods.

Sodium chloride has been shown to have no accelerating effect on oxidative rancidity in mayonnaise made from cottonseed oil (18), in herring oil (1), and in butterfat, lard, and pumpkin-seed oil (27). On the contrary, it was found to have an accelerating effect on oxidative rancidity in frozen pork (8,35,38), in cured pork (37), in bacon (9), in butter (4,5,10, 17.25,28,34), and in fish (1,6).

From a study on five series of butter consisting of 192 samples made and held under carefully controlled conditions for six years at 0 to −10°C., the relationship between the spoilage factors of NaCl, acid, both NaCl and acid, and natural milk spoilage enzymes was found to be 1:3:4:6, respectively (29). Commercial salt, when dispersed in the solid state in butterfat, exerted a catalytic effect on fat oxidation (12). This catalytic effect on fat oxidation by commercial

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[2] American Meat Institute Foundation Journal Paper No. 203.