TABLE I

MELTING POINTS, NEUTRALIZATION EQUIVALENTS AND ANALYSES OF ACIDS OF THE TYPE: RSCHCOOH (R′)

	M. p., °C.	Neutralization equivalent Calculated	Found	S % Calcd.	Found	Ba % (of salt) Calcd.	Found
R = $C_{12}H_{25}$-							
R′ = C_9H_{19}-	46–48	386.4	387.0[a]	8.28	8.03	15.13	15.14[a]
R = $C_{14}H_{29}$-							
R′ = C_2H_5-	38–39	316.4	318.0[a]	10.10	10.26[a]	...	...
R = $C_{16}H_{33}$-							
R′ = H-	73.5–74	316.4	316.5[a]	10.10	10.20	...	...
CH_3-	58–59	330.4	330.5[a]	9.70	9.82	...	...
C_3H_7-	47.5–49	358.3	357.8[a]	8.94	8.87[a]	...	...
C_4H_9-	48.5–49.5	372.3	372.0[a]	8.60	8.81	...	...
C_8H_{17}-	42–43	428.5	430.0[a]	7.46	7.27	13.86	13.70[a]
C_9H_{19}-	47–49	442.5	443.3[a]	7.24	6.79	13.46	13.49[a]
$C_{10}H_{21}$-	46–48	456.5	457.5[a]	7.00	6.95	13.10	13.28[a]
$C_{12}H_{25}$-	46–48	484.5	483.5[a]	6.62	6.45	12.44	12.60[a]
$C_{14}H_{29}$-	46–48	512.6	511.5[a]	6.26	5.97	11.83	11.63[a]

[a] Average of two analyses.

The writers are indebted to Dr. R. J. Anderson for suggesting the procedure followed in the preparation of the barium salts.

3. Analysis

(a) Sulfur and barium were determined gravimetrically by conventional methods.

(b) Neutralization equivalents were determined by titration of benzene solutions of the acids with .02 *N* alcoholic potassium hydroxide using thymolphthalein as an indicator.

Summary

1. Some new aliphatic acids of the type: RSCHCOOH (R′) with molecular weights above 300 have been synthesized as possible antiseptic agents.

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Thermal Decomposition of Lard

BY C. DONALD LARSEN AND HAROLD P. MORRIS

During the course of an investigation into the effects of ingested heated lard on the gastro-intestinal tract of the rat,[1] we studied some of the chemical changes which occurred in lard as a result of heating. It had been noted that our experimetal rats fared well on a diet containing 50% of unheated lard. Other groups of rats, which were fed diets in which the unheated lard was replaced by lard that had been heated up to and including 300°,[2] likewise consumed adequate quantities of food and grew reasonably well. On the other hand, when 50% of the diet consisted of lard that had been heated at 340–350°, the animals found the diet unpalatable, ate poorly and lost weight rapidly. It seemed probable, therefore, from the biological experiments, that more extensive chemical changes had occurred in the most drastically heated lard than in samples heated at lower temperatures. This assumption was subsequently verified.

Banzon[3] heated coconut oil at its boiling point (*ca.* 300°), in the presence of various catalysts, and reported the formation of much unsaponifiable material. A distillate was collected and a white crystalline powder was isolated from the unsaponifiable fraction. Banzon suggested that the material might be a ketone. Holleman and Koolhaas[4] confirmed the report of Banzon and extended the study of the unsaponifiable fraction obtained from both the distillate and from the residue of coconut oil heated at 300–320° with the aid of a reduced iron powder as catalyst. They reported the isolation of *n*-12-pentacosanone, which resulted from the pyrolysis of lauric and myristic acid glycerides, as well as considerable quantities of other ketonic material. Roffo[5] reported that the heating of lard to 350° for thirty minutes destroyed all of the cholesterol, to the extent that it was no longer precipitated by digitonin. Mauthner and Suida,[6] Veldstra,[7] Waterman and van Vlodrop,[8] and others, pyrolyzed cer-

(1) Morris, Larsen and Lippincott, *J. Natl. Cancer Inst.*, in press.

(2) All temperature values in this paper are on the centigrade scale.

(3) Banzon, *Philippine Agr.*, **25**, 817 (1937); **26**, 399 (1937).

(4) Holleman and Koolhaas, *Rec. trav. chim.*, **58**, 666 (1939).

(5) Roffo, *Bol. inst. med. exptl. estud. cancer*, **15**, 407 (1938).

(6) Mauthner and Suida, *Monatsh.*, **17**, 29 (1896).

(7) Veldstra, *Nature*, **144**, 246 (1939).

(8) Waterman and van Vlodrop, *Rec. trav. chim.*, **57**, 629 (1938).

TABLE I

ANALYSES OF HEATED LARD

Temp., °C.	Time, min.	I. N.[a] Whole lard	Free acid %	Free acid I. N.	Unsapon. %	Unsapon. I. N.	Sterol chromo[b]	(Mgm. %) ppt.[c]
Control lard		67.3[d]	0.36	80.1	0.26	73.0	114	108
200	30	65.4	0.47	75.0	0.24	73.8	107	102
250	30	64.6	0.47	73.8	0.33	74.6	107	97
300	30	64.2	1.15	59.7	0.47	75.5	136	68
300	60	62.1	1.99	51.7	0.55	75.6	138	41
300	120	60.9	3.49	50.4	0.74	76.5	145	28
350	30	55.6	23.10	44.9	8.87	78.7	153	00
350	60	54.7	29.40	44.2	14.10	82.3	156	00
350	150	53.2	43.40	42.2	18.70	87.0	158	00

[a] I. N. = Iodine Number. [b] Colorimetric values in terms of pure cholesterol. [c] Digitonin-precipitable sterol. [d] The data in this table in every case are the average of three to five determinations. Agreement on the average was ±1.2 for iodine numbers, ±1.1% for free acid, ±0.8% for unsaponifiable material, and ±8 for cholesterol values. In general, samples heated at less than 350° provided data which differed from the respective averages to a lesser degree. Free acid and unsaponifiable data from samples heated at 350° varied to a greater degree; maximum variations were ±3.4 and ±4.9, respectively.

tain esters of cholesterol and obtained 3,5-cholestadiene, together with varying quantities of isomers.

This paper deals with some of the changes which are found to occur in lard during heating, without added catalyst, at several temperatures between 200–350°, for periods of thirty to one hundred fifty minutes. We have limited our investigation to determinations of the increase of free fatty acids and unsaponifiable material, to iodine number determinations on these fractions, and on the whole fat from which they came, and to cholesterol analyses on the several unsaponifiable fractions. A preliminary fractionation of the unsaponifiable material has been made.

We have found that the non-catalyzed pyrolysis of the triglycerides of lard, as manifested by the increase of free acid and of unsaponifiable material, proceeds at a relatively low rate at temperatures as high as 300°. The rate of thermal degradation is quite uniformly accelerated as the temperature increases. Likewise, the extent of triglyceride decomposition, at any given temperature less than 350°, appears to be proportional to the time during which the elevated temperature is maintained. However, as a temperature of 350° is approached, the rate of pyrolysis of the triglycerides is very markedly accelerated, as is shown by the increase in the fatty acid and unsaponifiable fractions (Table I). The extent of pyrolysis at 350°, for periods of thirty, sixty and one hundred fifty minutes, does not appear to be related to the time factor, as 32% of the original lard is found in the form of free acids and unsaponifiable material after thirty minutes, 43.5% after sixty minutes, and 62% after one hundred fifty minutes, respectively. As our interest in the rate of decomposition of heated lard was secondary to studies of the quantities and types of reaction products in the pyrolyzed lard, this phase of the reaction was not investigated further.

It was found that the free acid fraction increased from 0.36% in stock lard to an average of 43.4% in samples heated at 350° for one hundred fifty minutes. Simultaneously, the unsaponifiable fraction increased from 0.26% to an average of 18.7% (Table I).

Iodine number values decreased from 80.1 in the free acids of stock lard to an average of 42.2 in the free acid fractions from the most vigorously heated samples. Conversely, it was found that iodine number values of the unsaponifiable fractions increased from 73.0 to 87.0 (Table I)

Cholesterol analyses revealed that approximately 40% of the original sterol was no longer precipitable with digitonin after lard had been heated at 300° for thirty minutes. After two hours at 300°, 75% of the sterol had been rendered inactive to digitonin. Heating at 350° for thirty minutes reduced the precipitable cholesterol to zero (Table I). Paradoxically, use of a colorimetric method revealed that as digitonin-precipitable cholesterol values decreased, colorimetric values increased. The increased chromogenicity of the unsaponifiable fractions from the most vigorously heated samples of lard was probably due, at least in part, to formation of the isomeric cholestadienes. We have found[9] that 3,5-cholestadiene develops about 70% more color than cholesterol under the conditions employed in our routine analyses.

Experimental

The lard used in these experiments was homogeneously rendered under carefully controlled conditions at one of the larger packing plants, and was of a quality equal or superior to the best grades of market lard. Prior to experimental use the lard was dried over calcium chloride. Samples of 150–160 g. were placed in a 250-cc. Pyrex distillation flask which was fitted with an air condenser and with a thermometer dipping into the molten lard. Samples heated at 300° or less fumed very little and the use of the condenser was not necessary. When samples were heated to 350° it was noted that fuming became pronounced at 320–330° and ebullition of volatile substances from the surface of the liquid began at about 340°. Most of this material condensed in the neck of the flask and very little escaped through the side-arm until 350° was reached. That which was trapped by the condenser was found to be princi-

(9) Unpublished data.

pally acrolein and water, together with small amounts of volatile fatty acids. The uncondensed portion of the volatilized material consisted for the most part of carbon dioxide together with traces of gaseous organic substances.

Free Fatty Acids.—Determinations of the free fatty acid content of heated or unheated lard were made by dissolving a suitable sample in petroleum ether (b. p. 30–45°) and extracting the free acids with a solution of 1% potassium hydroxide in 50% alcohol. The aqueous-alcohol solution of soaps was acidified and the liberated acids were extracted with petroleum ether. Suitable aliquots were evaporated to dryness under nitrogen and weighed.

Unsaponifiable Material.—Isolation of the unsaponifiable fraction from a sample of heated or unheated lard was accomplished in the usual manner. A sample of convenient size was boiled for one hour in 7 to 8 volumes of 10% alcoholic potassium hydroxide. Approximately half of the alcohol was removed by distillation under reduced pressure and the residual soap solution was diluted with one volume of water. The unsaponifiable material was recovered by repeatedly extracting the soap solution with petroleum ether. The several extracts were combined, partially concentrated, and washed with dilute alkali and water. The solvent was then completely removed under reduced pressure and the residue was dissolved in chloroform and the volume suitably adjusted. Aliquots were evaporated to dryness and weighed. Other aliquots were used for iodine number and cholesterol determinations.

Iodine Number.—Use was made of the pyridine–sulfate–dibromide method of Rosenmund and Kuhnhenn[10] as modified by Yasuda.[11]

Cholesterol.—Two standard methods were used for cholesterol determinations. The Liebermann–Burchard reaction, as modified by Bloor,[12] was used for the colorimetric assay of all chromogenic sterols present in a given sample. Suitable concentrations of purified cholesterol were used as standards. Comparisons were effected by use of a photoelectric colorimeter. Values obtained are recorded as units of pure cholesterol. Assays on comparable samples of unsaponifiable material, by the method of Schoenheimer and Sperry[13] (with slight modifications), furnished the data for the total digitonin-precipitable sterol. The intact cholesterol was precipitated with digitonin. The washed and dried digitonide was used for the development of color and the absorption values obtained were translated into cholesterol values with the aid of a standard cholesterol digitonide absorption curve. The latter method appeared to give a true picture of the fate of cholesterol *per se* in drastically heated lard.

(10) Rosenmund and Kuhnhenn, *Z. Untersuch. Nahr. Genussm.*, **46**, 154 (1923).

(11) Yasuda, *J. Biol. Chem.*, **94**, 401 (1931).

(12) Bloor, *ibid.*, **77**, 53 (1928).

(13) Schoenheimer and Sperry, *ibid.*, **106**, 745 (1934).

Preliminary Examination of Unsaponifiable Fraction.—A preliminary fractionation of the unsaponifiable material from lard heated at 350° was made. Approximately 50% of the unsaponifiable fraction solidified at room temperature and was separated from the liquid phase by centrifugation. The solid phase was dissolved in hot absolute alcohol from which, on cooling, the material crystallized in the form of crude leaflets, and was easily separated by filtration. The substance melts at 76–78° and is without doubt a mixture of aliphatic ketones derived principally from 16 and 18 carbon acids. Reaction of the ketonic mixture with hydroxylamine hydrochloride resulted in the formation of a colorless oil. A dilute solution of the oximes in alcohol, slightly acidified with acetic acid, resulted in regeneration of the ketones. Attempts to prepare a semicarbazone or a phenylhydrazone were unsuccessful. A more extensive study of the unsaponifiable fraction in general, and the ketonic components in particular, is contemplated.

Summary

Samples of lard have been heated at several temperatures between 200 and 350°, with variations in the length of the heating period.

The free acid and unsaponifiable fractions in lard increase uniformly with gradations in the temperature of heating up to 300°. As the temperature approaches 350°, decomposition of the lard, with formation of free acids, acrolein, carbon dioxide, water and unsaponifiable material, is greatly accelerated.

The iodine number of the free acid fraction from heated lard decreases as the temperature is increased and the total quantity of free acids is increased. The iodine number of the simultaneously formed unsaponifiable fraction becomes greater as the yield of unsaponifiable material becomes greater.

Cholesterol in lard, after heating to 350°, becomes incapable of forming a precipitate with digitonin. The chromogenicity of the unsaponifiable fraction increases as the cholesterol content decreases.

A preliminary examination of the unsaponifiable material from drastically heated lard gives evidence that it is principally ketonic in nature.

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