Rancidity in Indian Butterfats (Ghee)

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It has long been realized that the analytical characteristics of deteriorated butterfats depart considerably from those of the fresh fats. It is the more surprising, therefore, that the changes in butterfat, or ghee, during ordinary rancidification—in loosely corked containers exposed to diffused daylight—have not been more intensively studied. Elsdon, Taylor & Smith (1931) studied commercial butters for very short periods and found increased Polenske values, while for butterfat Godbole & Sadgopal (1936) found decreased Polenske and increased Reichert values. Curli (1939) and Das Gupta (1939) excluded air from their samples during storage, while Narasimhamurty (1941) used commercial ghee samples whose purity was open to suspicion. In the present study, sets of samples of genuine butterfat from three different localities were chosen, and stored under the first-mentioned, more or less 'normal' conditions. The samples were not chosen at random, but represent characteristic products, one group from Indian buffaloes the butterfats of which were of roughly normal Reichert value (high for a buffalo) and normal iodine value, another from buffaloes heavily fed with cottonseed, a practice common in many dry areas in India, and a third from cows in pasture, such as are produced in any well-kept dairy. It is possible, therefore, to draw comparisons between one set and another and also between individuals in the same group, a feature which will be appreciated during the discussion.

A study of the changes in the characteristics alone, while it enables certain conclusions to be drawn, would it was felt be somewhat restricted and it was decided therefore to isolate and study the free fatty acids produced by rancidity in the three batches of ghee. The products of spoilage of fats, as hitherto enumerated and sometimes isolated, include *inter alia* ketones, aldehydes, peroxides, hydroperoxides, ozonides, hydroxyacids and fatty acids. Whilst clearly the terms rancidity and acidity due to free fatty acids are not equivalent, it will be conceded that free fatty acids, especially over long periods as in the present instances, certainly form the largest proportion of the rancidity products, and may amount to as much as 20% of the rancid fat, while peroxides (calculated as oleic peroxide) may approach only 2% and aldehydes and ketones, according to Mundinger (1930), 'can be detected'.

The formation and composition of these fatty acids therefore represents a major factor in fat spoilage, especially in rendered fat where lipolytic complications may tend to be of subsidiary occurrence.

EXPERIMENTAL AND RESULTS

Methods

The three sets of Indian ghee chosen for study were: (1) eleven samples from buffaloes of the Agricultural College, Kirkee, initially of high Reichert and low iodine values, a characteristic sample of which had been analyzed earlier for fatty acid and glyceride composition by Achaya & Banerjee (1946); (2) ten samples from Porbandar buffaloes (heavily fed with cottonseed under arid conditions) of extremely low Reichert and high iodine value: a representative sample had been studied earlier; (3) twelve samples from cows of the Cattle Farm, Hosur, as representing normal, highgrade cow ghee.

All the products were in excellent condition when originally analyzed, but, when the present study was undertaken, had been maintained in storage under the conditions mentioned earlier for 3–4 years. The characteristics were determined in the usual way: acidities are expressed as acid values (mg. KOH/g. fat); peroxide values as ml. 0.002N- $\text{Na}_2\text{S}_2\text{O}_3$ /g. fat; and refractive indices in terms of butyrefractometer degrees at 40°.

After the characteristics had been determined, the individual samples in each lot were mixed and the free fatty acids extracted successively with boiling ethanol by thoroughly mixing, cooling till the fat solidified and drawing off the supernatant ethanol. Owing to the low concentration of total fatty acids present in the ethanol (maximum 7%) and to mutual solubility effects between the fatty acids there is little reason to fear that stearic or higher acids may thus have been lost by deposition with the neutral fat in the solid phase during the ethanol extractions. After five or six extractions (when typical acidities of the orders, 23, 11, 5.7, 4, 2.7, 1.8% were left in the separated fat phases), the ethanol extracts were mixed, exactly neutralized with conc. KOH, the ethanol driven off and the soaps thoroughly extracted with ether to remove any adventitious neutral fat. Tables 1 and 2 give a summary of the results obtained.

Analytical results

The changes in the analytical figures are in general extremely well defined, and very similar in the three groups. Of the thirty-three samples, only three show a fall (and these extremely small) in Reichert value. The increases in Polenske value are more striking, amounting sometimes to four times that of the fresh

fats but averaging about double. With a single exception the iodine values, as to be expected, showed diminution to varying extents which are roughly parallel with the production of free acidity in the individual specimens concerned. Large increases in the saponification values are almost always apparent, while the refractive indices vary erratically.

The acidities and peroxide values demand separate consideration. It is abundantly clear that, in advanced rancidity, the peroxide values are little indication of the extent of its progress as perceived organoleptically. A curious feature associated with these samples may be mentioned. Some of the Kirkee samples were coloured quite bright yellow, while the others were almost colourless, and it is in the former samples that the peroxide and acid values are very small. This would lead one to speculate on the possible anti-oxidative nature of the carotene in the specimens—further supported by the generally low acid and peroxide values of the bright yellow cow ghees from Hosur, and the contrasting high values of the extremely white Porbandar buffalo ghees.

value (usually also associated with large increases in peroxide value) are almost invariably accompanied by similarly large increases in Reichert, in Polenske and in saponification values and, most significantly, by correspondingly large falls in iodine value. The peroxide values are very small in actual amount (1–2 % of the total fat), and, since they are generally recognized in rancidity as precursors of further breakdown products, it is perhaps natural to expect them in quantities proportional to the fatty acids already present, fresh peroxide being formed as part of it was converted to further products. Increases in acidity run concurrently with losses in iodine value: in butterfat, the latter is a measure mainly of oleic glycerides, and it follows that oleic glycerides are therefore converted largely into free acids. Further evidence that this is the case is obtained by plotting the loss in iodine value of each sample against the acidity (calculated as a percentage of the mean molecular weight of the free acids (saponification equivalents) as shown in Table 2). The resulting smooth curves (Fig. 1) appear to be characteristic of an autocatalytic reaction, the agent being perhaps the peroxides initially formed or even the free acids

Table 1. *Changes in the analytical characteristics of ghees during development of rancidity*

Characteristic	Original samples		Rancid samples		Change due to rancidity	
	Range	Average	Range	Average	Range	Average
Kirkee, buffalo ghees, 11 samples, normal pasture feed						
Acid value	Negligible	Negligible	5.3– 52.3	24.9	+ 5.3– + 52.3	+ 24.9
Saponification value	223.9–239.3	228.9	227.5–261.0	243.6	- 2.2– + 33.5	+ 14.7
Iodine value	25.7– 31.1	28.9	6.9– 28.3	18.1	- 24.2– + 0.8	- 10.8
Butyrefractometer reading (40°)	39.2– 43.1	41.9	40.3– 42.6	41.7	- 1.6– + 1.3	- 0.2
Reichert value	25.7– 39.1	32.1	32.2– 40.7	36.4	- 0.6– + 12.3	+ 4.3
Polenske value	1.1– 2.7	1.7	1.7– 4.4	2.9	- 0.3– + 2.9	+ 1.2
Peroxide value	Negligible	Negligible	0.7– 71.6	34.7	+ 0.7– + 71.6	+ 34.7
Porbandar, buffalo ghees, 10 samples, high cottonseed feed						
Acid value	Negligible	Negligible	11.6– 24.1	18.2	+ 11.6– + 24.1	+ 18.2
Saponification value	200.8–224.0	213.3	213.0–250.2	226.9	- 2.6– + 29.5	+ 13.6
Iodine value	30.6– 39.9	35.4	14.0– 28.3	19.0	- 22.1– - 7.4	- 16.4
Butyrefractometer reading (40°)	43.8– 46.9	45.6	43.8– 46.4	45.1	- 1.4– + 0.9	- 0.5
Reichert value	14.5– 33.6	21.0	18.9– 37.0	24.9	- 0.7– + 5.8	+ 3.9
Polenske value	0.4– 2.4	1.1	1.1– 3.7	1.9	- 1.0– + 2.8	+ 0.8
Peroxide value	Negligible	Negligible	19.4–103.4	54.0	+ 19.4– +103.4	+ 54.0
Hosur, cow ghees, 12 samples, normal feed						
Acid value	Negligible	Negligible	5.3– 14.8	10.1	+ 5.3– + 14.8	+ 10.1
Saponification value	213.2–226.3	221.4	227.6–238.3	232.5	+ 4.5– + 22.6	+ 11.1
Iodine value	34.5– 39.2	37.1	22.2– 35.0	28.8	- 15.2– - 1.8	- 8.3
Butyrefractometer reading (40°)	43.8– 45.0	44.3	43.7– 45.2	44.3	- 1.1– + 0.7	Nil
Reichert value	23.8– 26.2	24.8	27.9– 30.2	28.9	+ 3.1– + 5.2	+ 4.1
Polenske value	1.2– 2.1	1.6	2.0– 3.0	2.5	+ 0.4– + 1.6	+ 0.9
Peroxide value	Negligible	Negligible	15.0– 48.5	32.5	+ 15.0– + 48.5	+ 32.5

The most outstanding feature of Table 1, however, is the marked parallelism between the alterations in each characteristic, suggesting that they all proceed from a single general cause. Large increases in acid

themselves. Davies (1941) studied somewhat empirically the free fatty acids of rancid butter and found them to be mainly composed of oleic and lower acids; while Rangappa & Banerjee (1946) found

broadly similar features in the acid fractions extracted from market samples of butterfat: it is often the case, however, that these are made from butter that

Table 2. *Free fatty acids from united samples of rancid ghees*

	Mixed rancid ghee	Mixed neutralized ghee	Free fatty acids isolated from rancid ghee
	Kirkee, buffalo ghee		
Appearance	Soft; heavy odour	Hard; bland odour	Soft; yellow; crystalline solid
Melting point (°)	34.3	40.3	32.6
Solidification point (°)	27.8	32.8	31.6
Butyrefractometer reading (40°)	41.7	42.6	29.4
Acid value	23.0	1.8	—
Saponification value	243.6	221.4	320.3
Saponification equivalent	230.2	253.4	175.2
Iodine value	19.1	15.9	26.4
Reichert value	35.9	26.9	119.8
Polenske value	3.1	1.7	13.5
Peroxide value	30.7	16.9	—

Porbandar, buffalo ghee

	Pasty; acid odour	Hard and granular; bland odour	Just solid; amber coloured; crystalline
Appearance			
Melting point (°)	38.7	39.2	32.7
Solidification point (°)	34.5	36.0	29.6
Butyrefractometer reading (40°)	44.4	45.2	30.6
Acid value	23.1	4.6	—
Saponification value	237.1	230.3	294.4
Saponification equivalent	236.6	243.5	190.6
Iodine value	20.6	20.3	22.5
Reichert value	28.8	24.9	49.3
Polenske value	2.7	1.3	13.2
Peroxide value	28.5	17.7	—

Hosur, cow ghee

	Pasty; rancid but not acid odour	Soft; faintly granular; bland odour	Very soft; dark amber in colour; crystalline
Appearance			
Melting point (°)	35.8	34.2	28.0
Solidification point (°)	36.4	36.6	28.0
Butyrefractometer reading (40°)	43.7	44.4	37.1
Acid value	10.6	3.1	—
Saponification value	232.1	228.8	293.6
Saponification equivalent	241.6	245.3	191.1
Iodine value	29.6	30.0	29.1
Reichert value	28.8	26.5	67.6
Polenske value	2.5	2.1	13.0
Peroxide value	32.7	26.8	—

has deteriorated considerably so that the characteristic rancidity products of the latter are liable to be carried over. Again, the Reichert and Polenske

values of the de-acidified fats studied in the present work were in all three cases extremely close to the original values for these fats when fresh, indicating that these glycerides had largely remained intact. All these features appear to point to one fact: a mainly

Fig. 1. Rise of acidity accompanying loss in iodine value during rancidity.

oxidative mechanism producing free acidity in butterfat during spoilage, as opposed to a lipolytic one (possibly somewhat selective towards oleic and lower fatty glycerides) in butter.

Detailed study of the free fatty acids

The free fatty acids (thus isolated in the form of dry soaps) were now analyzed for component fatty acids; the Kirkee soaps were divided into two lots and analyzed using the lead-salt separation technique and the low-temperature crystallization procedure (using 10 ml. of ether/g. of acids at -45°), respectively, for the partition of the acids non-volatile in steam (Hilditch, 1947*a*) the results being calculated on the basis of normal straight-chain fatty acidity. The analyses differed substantially and whilst this may partly be due to the small amounts available for analysis, a more important cause appeared from subsequent work to lie in the presence of short-chain dicarboxylic acids and *n*-octane-1-carboxylic acid (produced as scission products of oxidation from oleoglycerides, the presence of which was not at first realized). The dicarboxylic acids appeared in both the soluble and deposited portions during the lead-salt separation, whereas the crystallization procedure gave a more straightforward separation and was consequently employed for the other two analyses. Details and results obtained by the alternative lead-salt method of separation will not therefore be recorded here.

When the presence of significant proportions of *n*-octane-1-carboxylic acid and also, apparently, dicarboxylic acids was appreciated, an attempt was made to identify these and also, by modifying the ester fractionation procedure, to obtain an approximate measure of the amounts present. For this

purpose the following methyl ester fractions were mixed and carefully refractionated through the electrically heated and packed column: from Porbandar rancid buffalo ghee—ester fractions from the steam-volatile acid group (saponification equiv. 134.9–182.3) and from the acids soluble in ether at -45° (saponification equiv. 128.1–164.3); from Hosur rancid cow ghee—ester fractions from the steam-volatile acid group (saponification equiv. 131.2–198.8) and from the acids soluble in ether at -45° (saponification equiv. 129.7–178.1). The boiling points (at about 0.2 mm. pressure) rose steadily from 54 to 124° , but the equivalents of the fractions obtained were, in order of increasing boiling point: 148.3, 153.6, 164.2, 169.4, 133.4, 119.8, 118.4, 131.8, 170.8, 177.0, 201.0 and 243.3. It was evident that esters of dibasic acids were present (e.g. dimethyl azelate has boiling point 140 – $141^\circ/9$ mm.). These were therefore removed by washing the remixed free acids with light petroleum, filtering, and isolating any dissolved dibasic acids by cooling the monobasic acids several times in light petroleum or ether solutions at temperatures down to -20° . Finally there were obtained 12.9 g. of crude dibasic acids and 12.8 g. of monobasic acids. The latter were re-esterified and fractionally distilled, and now showed no discontinuity in the sequence of saponification equivalents which rose steadily as follows: 162.1, 169.1, 169.2, 180.8, 188.2, 198.1, 212.6, 230.1 and 284.4. The determined figures for the acids present in this group of esters fractionated after removal of the dibasic acids, were: *n*-heptane-1-carboxylic, 7.6; *n*-octane-1-carboxylic, 18.1; *n*-nonane-1-carboxylic, 22.5; lauric, 29.5; myristic, 8.8; palmitic, 6.2 and stearic, 7.3 % (w/w), whilst typical estimates of the total esters (including dicarboxylic esters calculated as dimethyl azelate) present in two of the mixed esters were as follows: *n*-pentane-1-carboxylic, 2.3, 0.8; *n*-heptane-1-carboxylic, 14.0, 7.5; *n*-octane-1-carboxylic, 18.5, 19.9; azelaic, 29.8, 31.7; *n*-nonane-1-carboxylic, 22.8, 22.9; lauric, 7.5, 8.3; myristic, 4.8, 8.9; and palmitic, 0.3 % (w/w).

By crystallizing the crude dibasic acids (cf. above) repeatedly from CHCl_3 at room temperature they were ultimately separated into two fractions, (i) about 80 % of the crude acids, which now melted at 86 – 96° and had an equivalent of 95.4 (azelaic acid 94.0), and (ii) about 20 % of acids which melted at 106 – 108° and had an equivalent of 81.8. It therefore appeared that the greater part of the dicarboxylic acids consisted of azelaic acid, $\text{COOH}(\text{CH}_2)_7\text{COOH}$, but it is possible that small proportions of dibasic acids of higher (?sebacic) and lower mol. wt. than azelaic acid were also present amongst the products of rancidity.

The presence of *n*-octane-1-carboxylic acid was confirmed by its identification as the *p*-toluidide, this derivative being selected because of the convenient discriminate melting points in this range of the homologous series. For comparison, the *p*-toluidides of authentic specimens of butyric, *n*-pentane-*n*-hexane-, *n*-heptane-, *n*-octane- and *n*-nonane-1-carboxylic acids were also prepared. The sources of these acids were as follows: butyric and *n*-hexane-1-carboxylic acids were purchased from commercial sources (equiv. 88.8 and 128.8, respectively). *n*-Pentane- and *n*-octane-1-carboxylic acids were prepared by oxidizing a concentrate of methyl oleate and linoleate (iodine value 103.1) with KMnO_4 in acetone, isolating by distillation in steam the monobasic acids formed, and distilling the latter through a fractionating column when *n*-pentane and *n*-octane-1-carboxylic acid fractions were obtained in a state of purity (equiv. 116.0 and 157.6, respectively). *n*-Heptane- and *n*-nonane-1-carboxylic acids were obtained from the respective methyl esters iso-

lated by fractional distillation of the mixed methyl esters of coconut oil acids (equiv. 145.8 and 168.5, respectively).

Table 3. *p*-Toluidides of lower normal fatty acids

Acid used	Equivalent of acid		Melting point of <i>p</i> -toluidide ($^\circ$)		
	Found	Calc.	Found (present work)	Robertson	
				(1908)	(1919)
<i>n</i> -Butyric	88.8	88.0	74.5–75.5	74	75
<i>n</i> -Pentane-1-carboxylic	116.0	116.0	71.5–72.5 (rising)	75	73
<i>n</i> -Hexane-1-carboxylic	128.8	130.0	78.5–79.5	80	80
<i>n</i> -Heptane-1-carboxylic	145.8	144.0	68.0–68.5	67	70
<i>n</i> -Octane-1-carboxylic	157.6	158.0	83.0	81	84
<i>n</i> -Nonane-1-carboxylic	168.5	172.0	76.5–78.0	80	78

The fatty acids (about 1.0 g.) were refluxed under water condenser with 100 % excess of SOCl_2 for 1 hr., and excess reagent removed by gentle suction for 15 min.: 100 ml. of dry ether were now added to the mixture, 2.5 mol. of *p*-toluidine introduced and washed in with a little ether. A bulky precipitate of *p*-toluidine hydrochloride immediately formed; the whole was vigorously refluxed for 2 hr., more ether being added if necessary. The contents of the flask were washed with water and dilute HCl in a separating funnel, then with dilute KOH and finally with water. The extracts were dried, filtered, the product refluxed in hot ethanol with animal charcoal and crystallized from pure methanol or ethanol. Table 3 gives a summary of the properties of the *p*-toluidides prepared from the authentic acids. The products were highly crystalline compounds, but even after repeated crystallization still melted over a range of about 1 – 2° ; this, however, is not a drawback for the present purpose since the melting points of successive members are well apart. The melting points compare well with those recorded earlier in the literature (Robertson, 1908, 1919) except for the *n*-pentane-1-carboxylic derivative which was available in quantities too small for further crystallization, and was probably still impure. The present starting materials were in general probably of greater purity than those available to Robertson (1908, 1919).

From the ester fractions obtained after the removal of dibasic from monobasic acids (cf. col. 1) two fractions were chosen for preparation of derivatives, their respective saponification equivalents being 169.2 and 188.9 (cf. methyl *n*-octane-1-carboxylate, 172; methyl *n*-nonane-1-carboxylate, 186). The derivatives were obtained in theoretical yield, and after three crystallizations from ethanol, each gave highly crystalline products melting at 83.0 and 74.5° , respectively, undepressed on mixing with the corresponding pure toluidides and pointing unmistakably to the presence of *n*-octane- and *n*-nonane-1-carboxylic acids in the original fatty acids.

Another point of interest was to study whether fatty acids of lower molecular weight than butyric acid were produced during the course of oxidative rancidity. This was investigated by two methods: first, by isolating the acids from the titrated soaps in the ether-extracted aqueous solution from

the steam distillate. These soaps were evaporated to dryness on a water bath and then on a sand bath at 150°, and finally kept in a desiccator until required for use. They were then distilled with dry H_3PO_4 (preheated to 150° for 3 hr.), and the distillate stood over anhydrous Na_2SO_4 for several days. About 0.8 g. of these acids was now fractionally distilled in a microfractionation apparatus and yielded fractions as follows:

Wt. (g.)	0.0883	0.0812	0.0838	0.0738
Equiv.	68.3	64.2	85.0	83.1
Wt. (g.)	0.0379	0.2200	0.1803	
Equiv.	84.0	105.3	97.3	

The first two fractions clearly contained acetic acid (equiv. 60), the remainder had acid equivalents corresponding with butyric (88.0) or mixtures of this with *n*-pentane-1-carboxylic (116.0) acid; the proportions of acetic: butyric are of the order 1:3.

The second method used was a chromatographic separation by the procedures of Martin & Synge (1941), Elsdon (1946), and Rawsey & Patterson (1946), employing moist silica gel as adsorbent and $CHCl_3$ containing *n*-butanol as eluent. Two bands were obtained with ease using a 1% butanol- $CHCl_3$ eluent, and when this was followed by 5% butanol- $CHCl_3$, a third band also appeared, but tended to become diffuse as it moved down the column. The silica gel was therefore removed in zones, placed in water, and, after aeration to remove $CHCl_3$, was gently steam distilled, the distillates titrated and calculated to acetic, butyric and *n*-pentane-1-carboxylic acids respectively, when the relative proportions by weight were found to be 2:6:3. This accords extremely well with the proportions 1:3 for acetic: butyric obtained by the former method.

It should be pointed out, however, that these results do

not demonstrate unequivocally that acetic acid is one of the products of a process of oxidative rancidity, since the quantities observed were so small that the possibility that the acetic acid arises adventitiously (e.g. from traces of acetic esters still left in the ethanol used in the course of the work in spite of drastic purification) must not be overlooked.

In the course of the above work, therefore, the following have been established: (i) The discrepancy between the lead-salt method of analysis and the low-temperature crystallization technique leading to the preferred use of the latter subsequently. (ii) The proof of the presence of dibasic acids, mainly azelaic, but with traces of both higher and lower homologues. (iii) The clear characterization of *n*-octane- and *n*-nonane-1-carboxylic acids by the preparation of their *p*-toluidide derivatives, with the incidental preparation of reference *p*-toluidides of several saturated fatty acids. (iv) The presence of acetic acid (though perhaps of adventitious origin) in the free fatty acids, as demonstrated by fractional distillation and by chromatographic separation.

DISCUSSION

Table 4 shows the results of the analyses of the free fatty acids of the rancid ghees. In the analyses of the Porbandar and Hosur samples it has been possible to make an approximate allowance for the *n*-octane-1-carboxylic and dibasic (as azelaic) acids which were shown to be present. Unfortunately shortage of material prevented this correction in the case of the Kirkee fatty acids.

Table 4. Composition of free fatty acids from rancid ghees

	Percentage (w/w)			Percentage (molar)		
	Kirkee (U)	Porbandar (C)	Hosur (C)	Kirkee (U)	Porbandar (C)	Hosur (C)
Saturated:						
Acetic	?	0.6	?	?	1.9	?
Butyric	10.9	3.5	4.0	21.5	7.5	8.7
Caproic	13.9	4.6	2.8	20.8	7.5	4.6
Caprylic	9.7	4.3	5.2	11.6	5.7	6.9
<i>n</i> -Octane-1-carboxylic	?	6.1	7.4	?	7.4	9.0
Capric	4.7	9.0	9.8	4.8	9.9	10.9
Lauric	4.3	3.0	4.0	3.7	2.9	3.8
Myristic	4.5	6.8	4.1	3.4	5.7	3.5
Palmitic	20.0	15.4	9.9	13.5	11.4	7.4
Stearic	3.1	4.3	1.3	1.9	2.9	0.9
Arachidic	—	—	0.8	—	—	0.5
Azelaic	?	9.4	11.4	?	9.5	11.5
Unsaturated:						
Nonene-1-carboxylic	0.8	0.9	0.7	0.8	1.1	0.8
Undecene-1-carboxylic	0.4	1.3	0.3	0.3	1.2	0.3
Tridecene-1-carboxylic	1.8	0.6	1.0	1.4	0.5	0.8
Pentadecene-1-carboxylic	6.7	9.1	10.2	4.5	6.8	7.7
Oleic	3.6	3.3	6.1	2.2	2.2	4.1
Unsaturated residues	15.6	17.8	21.0	9.6	15.9	18.6
Unsaturated residues:						
Equivalent		Kirkee	Porbandar	Hosur		
Iodine value		224.7	213.2	215.2		
		68.3	53.9	59.3		

U = Uncorrected for acetic, *n*-octane-1-carboxylic and azelaic acids. C = Corrected for acetic (Porbandar only), *n*-octane-1-carboxylic and azelaic acids (Kirkee and Porbandar).

The comparatively small amounts of experimental material available, together with the complexity of the fatty acid mixtures and the complication introduced by the presence of some dicarboxylic acid, causes the figures in Table 4 to be only broadly indicative in character. Nevertheless, there is considerable general resemblance between the proportions of the various component acids, which are apparently largely independent of the more widely variable nature of many of the acids in the three corresponding original ghees (cf. Achaya & Banerjee, 1946). Thus, the Porbandar samples were buffalo ghees of very low Reichert (14–20) and high iodine (30–40) value, high stearic and low palmitic acid content, in each of these respects differing from the Kirkee samples. The data in Table 4 thus suggest that the products of free acid and oxidative rancidity from all three ghees are much the same in type, especially the relative proportions of the lower saturated acids.

Characteristic features of these free acid fractions from the products of rancidity which merit some further notice are: (i) the presence of 10–20 % of unsaturated, non-volatile residues of low equivalent (210–225) and low iodine number (50–60) which account for about half the unsaturation of the total free acids; (ii) the presence of normal saturated acids from butyric to *n*-nonane-1-carboxylic in similar proportions (averaging about 8 % in each case); (iii) the presence of *n*-octane-1-carboxylic and dibasic (mainly azelaic) acids; (iv) the presence of about 10 % of palmitic acid; and (v) the absence of oleic acid in any great quantity, and the presence of fragments of lower unsaturated acids of indefinite structure.

(i) The unsaturated residues are clearly polymers of some kind as evident from their low iodine values and equivalents and their lack of volatility even under extreme conditions. The basis of these residues is evidently some product derived from oleic acid. Farmer (1942) first demonstrated that atmospheric oxidation of the oleic glyceride begins with the formation of a hydroperoxide group, $-\text{CH}(\text{O}.\text{OH}).\text{CH}:\text{CH}-$, which on further oxidation of the double bond forms short-chain fatty acids. In the view of Hilditch (1947*b*) the mechanism tends to proceed in the following sequence: loose attachment of oxygen at an ethenoid linkage, electronic displacements in the system thus produced, leading to the loosening of a hydrogen atom from an adjacent methylene group and the final formation of a hydroperoxide of the type shown above, the double bond having meantime shifted one step along the carbon framework. These hydroperoxides could conceivably polymerize with ease to produce partly unsaturated polymers. Moreover, the small percentage (up to 4) of linoleic glycerides present in ghee probably acts as a starter for the

whole mechanism by the formation of highly reactive conjugated unsaturated products. Polymerization occurring to such a marked extent in a relatively saturated fat does not appear to have been recorded previously.

(ii) and (iii). It was pointed out earlier from a series of observations that the lower saturated acids are probably produced largely from the oxidation of oleic glycerides. The formation of roughly similar amounts of the acids is explicable in terms of the above theory whereby the double bond apparently shifts during autoxidation: one could conceivably continue the process further along the carbon chain, and couple it with lipolysis at any stage; the observed presence, too, of acids probably mono-unsaturated, and of low molecular weight (calculated here as nonene-, undecene-1-carboxylic acids, etc.) in amounts much larger than in the original butterfats would again be possible on a similar hypothesis, while the production of azelaic acid (clearly much simple double bond scission occurs) and its higher and lower homologues would follow from hydrolysis of unsaturated intermediate products. The small amount of oleic acid in the free fatty acids hardly calls for explanation since it is even more vulnerable to attack in the free form, if produced, than as a glyceride.

(iv) The presence of 10 % of palmitic acid can hardly be traced to oleic acid, for even though saturation and chain shortening have been suggested in consequence of certain *in vivo* experiments, these are very unlikely in the present instance to have occurred concurrently with preponderating oxidation. Clearly lipolysis of glycerides can occur to a certain extent even in a rendered (sterile) butterfat, probably from subsequent enzymic contamination during storage, but it is apparent from the experiments now recorded that oxidation products predominate in the end products of rancidity.

Finally, the low saponification equivalents of the total free fatty acids (170–200) reveal that a considerable error may be introduced when expressing the free fatty acidity of ghee 'as % oleic acid', as is often the case. It is suggested that either the acid value, which is independent of the nature of the free acidity, be used, or else that a figure of, say, 200 be taken as approximating to the average equivalent of the free acids normally produced during rancidity.

SUMMARY

1. A study has been made of three characteristic sets of ghee (Indian butterfat), comprising about a dozen very similar samples in each, after maintenance in loosely corked bottles at temperatures between 15 and 20° over a period of 3 years.

2. Changes in the analytical constants were strikingly similar—increases in Reichert value, marked increases in Polenske and saponification

values, losses in iodine value, and rather erratic changes in refractive index.

3. Marked parallelisms between the alterations of the characteristics for any one sample suggested that these alterations proceed from a single general cause, most probably aerial oxidation. Increases in acidity, in particular, exactly paralleled loss in iodine value for any particular batch of samples, suggesting an essentially oxidative mechanism producing free acidity in butterfat as opposed to a mainly lipolytic one in butter.

4. The mixed free fatty acids from each batch of pooled rancid samples have been extracted thoroughly with ethanol and analyzed for component acids. Important features were: the presence of *n*-octane-1-carboxylic acid to the extent of 7–9 mol. % (proved by the preparation of its *p*-toluidide derivative, a series of such derivatives from pure fatty acids being also incidentally prepared for reference purposes) and of normal homologous saturated acids from butyric to *n*-nonane-1-carboxylic in roughly similar amounts; the, perhaps adventitious, presence of acetic acid (as shown both by chromato-

graphic and direct fractionation methods); the occurrence of azelaic acid, with traces of unidentified higher and lower homologous dicarboxylic acids; the presence of about 10 % of palmitic acid; the absence of any great quantity of oleic acid, but the presence of fragments of uncharacterized lower unsaturated acids in small amounts, and of non-volatile residues of low equivalent and low iodine value to the extent of 10–20 % (accounting for nearly half of the total unsaturation).

5. Most of the above features are considered to be explicable as resulting from the autoxidation, probably as a glyceride, of oleic acid by recently proposed mechanisms. Polymerization also emerges as an important feature of rancidification in these comparatively saturated fats. Glyceride lipolysis also occurs, but to smaller extents than the two effects just enumerated.

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The Use of the Tyrosine Apodecarboxylase of *Streptococcus faecalis* R for the Estimation of Codecarboxylase

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The bacterial L-lysine and L-tyrosine decarboxylases have been shown to contain a coenzyme known as codecarboxylase; this substance was found to be widely distributed in nature (Gale & Epps, 1944a, b; Epps, 1944). Gale & Epps have used the apoenzyme of the L-tyrosine decarboxylase of *Streptococcus faecalis* for the estimation of codecarboxylase; the rate of decarboxylation of tyrosine was a function of the amount of codecarboxylase added; the apoen-

zyme was prepared by the dissociation of the holoenzyme. A method of estimating codecarboxylase by means of tyrosine apodecarboxylase has also been described by Umbreit, Bellamy & Gunsalus (1945). They used, as source of apoenzyme, suspensions of freeze-dried cells of *Strep. faecalis* R, which had been grown in a vitamin B₆-free medium. Such preparations also contained an enzyme system which formed codecarboxylase from pyridoxal and adenosinetri-