The Component Fatty Acids of *Penicillium spinulosum* Fat

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The fatty acid ingredients in fat synthesis in *Aspergillus nidulans* and in *Penicillium lilacinum* have been identified and estimated by Singh, Walker & Meara (1955) and by Singh, Shah & Walker (1956a). *Penicillium spinulosum* produces fat in high yield when grown in a chemically defined glucose medium (Garrido & Walker, 1953; Garrido, Woodbine & Walker, 1953) or in a similar medium in which sucrose is the source of carbon (Gad & Walker, 1954), and, judging from the results of a preliminary nutritional trial, this fat does not seem to be toxic to rats (personal communication from Dr T. Moore, Dunn Nutritional Laboratory, Cambridge). More extensive trials of this fat as food are planned and its fatty acid composition has therefore been determined.

EXPERIMENTAL

The strain of *P. spinulosum* was isolated in this Laboratory and its identity was confirmed at the Centraalbureau voor Schimmelcultures, Baarn, Holland (personal communication). It was grown in a medium which contained (g./l.): $\text{NaH}_2\text{PO}_4 \cdot 2\text{H}_2\text{O}$, 0.270; $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$, 0.090; K_2SO_4 , 0.082; $\text{ZnSO}_4 \cdot 7\text{H}_2\text{O}$, 0.013; $\text{FeCl}_3 \cdot 6\text{H}_2\text{O}$, 0.040; NH_4Cl , 3.20; glucose, 100. These constituents were made up with tap water and the pH was adjusted with NaOH to 6.8. This medium (20 l.) was sterilized in steam at 10 lb./in.² for 15 min., cooled and introduced aseptically into a stainless-steel fermentation vessel which had already been sterilized by a current of steam. This vessel was fitted with an impeller geared to revolve at 500 rev./min. After inoculation of the medium with a suspension of spores, stirring was commenced and a stream of air (10 l./min.) was led through the medium for 7 days at 27°. The contents of the vessel were then drawn off and the whole (mycelium and

metabolism solution) was sterilized at 100° for 10 min. The mycelial tissue was separated by filtration, washed with hot water, pressed, spread on filter paper, dried for 2 days in a current of air at 27° and dried finally for 3 hr. in an oven at 60°. The material was then ground in a large coffee-mill, placed in a Soxhlet apparatus and extracted with light petroleum (b.p. 40–60°). After removal of the solvent, 125 g. of fat was obtained from 300 g. of dried felt. The fat was a reddish yellow liquid which, on examination according to British Standard Methods of Analysis of Oils and Fats (1950; B.S. 684), gave the following figures: saponification equivalent, 294.2; iodine value, 81.1; free fatty acids (as oleic acid), 5.8%; unsaponifiable matter, 1.4%. The greater part (120 g.) of the fat was saponified with 750 ml. of ethanol containing 6% (w/v) of KOH.

After removal of the bulk of the ethanol by distillation, the soaps were taken up in water and unsaponifiable matter was removed by ether. To the aqueous solution (600 ml.), 250 ml. of 4N- H_2SO_4 was added and the liberated component acids were taken up in ether, washed, dried (104.91 g.) and subjected to low-temperature crystallization by the procedure of Hilditch (1956). A solution (10%, w/v) of the mixed fatty acids in acetone was cooled in a bath of ethanol-solid CO_2 to -72° and maintained at that temperature until crystals separated. After 6 hr. these were filtered off with continued strong cooling, the compounds remaining in solution at this temperature constituting group D. The separated crystals were then recrystallized from solution (10%, w/v) in acetone by cooling to -40°. This afforded a soluble group (C), and an insoluble group which was recrystallized from solution (10%, w/v) in ether by cooling to -25°, whereby a soluble group (B) was separated from an insoluble group (A) (Table 1). Spectrophotometric determinations on groups A, B, C and D isomerized under standard conditions (Hilditch, Riley & Patel, 1951) after removal of unsaponifiable matter afforded the further data shown in Table 1.

Table 1. Fractionation of mixed fatty acids of *Penicillium spinulosum* fat by low-temperature crystallization

For explanation of groups A, B, C and D, see text.

Group	Conditions	Wt. (g.)	Percentage (w/w)	Iodine value	$E_{1\text{cm}}^{1\%}$ (234 mμ)*	$E_{1\text{cm}}^{1\%}$ (268 mμ)
A	Insoluble in 10 vol. of acetone at -40° and in 10 vol. of ether at -25°	29.50	28.1	7.9	—	—
B	Insoluble in 10 vol. of acetone at -40°, soluble in 10 vol. of ether at -25°	22.60	21.5	72.6	14.5	—
C	Soluble in 10 vol. of acetone at -40°	26.93	25.7	101.5	151	—
D	Soluble in 10 vol. of acetone at -72°	25.88	24.7	154.6	641	6

* After isomerization with alkali.

Table 2. Fractionation of methyl esters of acids of groups A, B, C and D

Fraction	Wt. (g.)	Saponification equivalent	Iodine value
Methyl esters of acids A			
A ₁	0.93	271.8	2.2
A ₂	1.42	275.5	2.7
A ₃	1.96	275.8	3.8
A ₄	1.65	277.7	2.4
A ₅	1.97	278.7	2.7
A ₆	1.86	279.5	4.9
A ₇	1.71	281.1	8.7
A ₈	1.90	291.2	9.6
A ₉	2.49	291.5	10.6
A ₁₀	1.06	299.5	11.6
A ₁₁	1.85	303.4	11.9
A ₁₂	1.87	305.0	13.5
Methyl esters of acids B			
B ₁	1.66	273.5	4.6
B ₂	1.93	286.6	63.4
B ₃	1.59	284.4	67.6
B ₄	1.73	289.5	71.3
B ₅	1.57	290.1	72.1
B ₆	1.73	292.2	73.7
B ₇	1.27	293.7	76.5
B ₈	1.81	295.5	77.6
B ₉	1.40	297.5	77.0
Methyl esters of acids C			
C ₁	1.82	289.3	91.3
C ₂	2.10	292.5	94.7
C ₃	2.03	292.3	97.5
C ₄	2.04	295.7	99.2
C ₅	2.22	295.8	98.2
C ₆	2.10	295.9	99.3
C ₇	3.34	298.0	99.0
C ₈	2.03	301.1	98.0
C ₉	1.40	301.1	98.0
Methyl esters of acids D			
D ₁	1.17	272.0	120.0
D ₂	2.27	288.0	132.4
D ₃	1.66	293.0	150.6
D ₄	2.67	293.7	152.3
D ₅	3.29	293.5	153.0
D ₆	2.18	294.9	152.8
D ₇	3.74	295.2	152.0
D ₈	0.98	311.0	141.1

Determination of component fatty acids

The different groups A, B, C and D were esterified separately (Bjarnason & Meara, 1944) and fractionally distilled under reduced pressure (0.1 mm. Hg) through an electrically heated column of the type described by Longenecker (1937). By taking suitably small fractions of the distillate it was possible to obtain not more than two homologous saturated acids and two unsaturated acids of the same series in each fraction. Table 2 shows the saponification equivalent and iodine value of each fraction. From the analytical data obtained for each ester fraction and from the appropriate spectroscopic data, its composition and thence that of each acid group and ultimately that of the whole fat was then ascertained (Table 3) in accordance with the general principles which Hilditch (1956) enunciated.

Identification of fatty acids

Specimens of free acids regenerated from fractions A₁–A₉ were recrystallized from ethanol, when each lot of crystals had m.p. 61.7–63.0° alone or when mixed with pure palmitic acid. From the saturated acids liberated from fractions A₈–A₁₁, after oxidation according to Lapworth & Mottram (1925), an acid was obtained of m.p. 69.3–69.9° undepressed by admixture with stearic acid of m.p. 70°. The presence of oleic acid in acids recovered from fractions B₄–B₉ and C₁–C₈ was established by conversion into 9:10-dihydroxystearic acid which, after crystallization from hot ethyl acetate, melted at 129.3–131.5°, alone or in admixture with authentic 9:10-dihydroxystearic acid of m.p. 131°. Fractions D₃–D₇ afforded linoleic acid, which was characterized by bromination in light petroleum (b.p. 40–60°), whereby it yielded tetrabromostearic acid, m.p. 113.2°, alone or when mixed with an authentic specimen of m.p. 114°.

DISCUSSION

The relatively high content of free fatty acids often noted in the fats of micro-organisms, particularly yeasts, would be a drawback if it were desired to incorporate such fats in feeding-stuffs. *P. spinulosum* fat has a higher free fatty acid content (5.8% as oleic acid) than any of the fats of five other moulds studied in these Laboratories

Table 3. Component fatty acids of *Penicillium spinulosum* fat

	Acid groups				Total fatty acids, excluding unsaponifiable material (% w/w)
	A (28.1%)* (%)†	B (21.5%)* (%)†	C (25.7%)* (%)†	D (24.7%)* (%)†	
Palmitic acid	46.3	19.5	2.3	1.2	18.0
Stearic acid	40.9	1.8	0.4	—	11.9
Arachidic acid	3.9	0.5	0.8	—	1.4
Hexadecenoic acid	—	7.4	3.5	4.9	3.8
Oleic acid	8.9	69.4	76.6	24.7	43.3
Linoleic acid	—	1.4	16.3	67.1	21.1
Linolenic acid	—	—	—	1.2	0.3
Eicosenoic acid	—	—	—	0.8	0.2
Unsaponifiable matter	—	—	0.1	0.1	—

* Groups as % (w/w) of total acids.

† Component acids as % (w/w) of group.

(Singh *et al.* 1955; Singh *et al.* 1956a; Singh, Shah & Walker, 1956b; Singh, 1957; Singh, Philip & Walker, 1957). On the other hand, even this value is much lower than the corresponding values reported for yeast fats. For example, the free acidity of *Rhodotorula gracilis* fat is 18% (Holmberg, 1948) and that of the fat of yeast strain no. 72 is 33% (Hilditch & Shrivastava, 1948).

Table 4 permits comparisons of the analytical data for the fat of *P. spinulosum* with the figures for the fats of *A. nidulans* and *P. lilacinum*. Data derived from analysis of palm oil (Bank, Dean & Hilditch, 1935), olive oil (Gunde & Hilditch, 1940) and groundnut oil (Crawford & Hilditch, 1950) are also shown for comparison with the figures relating to the mould fats, because one of the objects of the present work was to find whether mould fats could be used in special cases as substitutes for vegetable oils.

Singh *et al.* (1955) drew attention to the fact that, in the triglyceride portion of fats of microbiological origin reported up to that time, 'the palmitic acid content, while being somewhat variable, constitutes about 70% of the saturated acids present, the remaining acids consisting of stearic acid and smaller amounts of myristic, and the higher arachidic, behenic and lignoceric acids'. Further, these authors proceeded to show that in *A. nidulans* fat the palmitic acid content was only 5 units % higher than the stearic acid content, palmitic acid constituting 52.7% of the total saturated fatty acids. A similar quantitative relationship between the palmitic acid and stearic acid components of a fat is now seen in *P. spinulosum* fat, for the quantity of palmitic acid incorporated into it is only 6.1 units % higher than the quantity of stearic acid combined in this fat; the palmitic acid component constitutes 57.7% of the total saturated fatty acids. Accordingly, *P. spinulosum* fat affords another example of a mould fat which resembles to some extent the seed fats of certain tropical plants, e.g. *Madhuca latifolia* (Hilditch & Ichaporla, 1938). Whereas stearic acid thus constitutes more than one-third of the total saturated acids which are combined in the glycerides of *P. spinulosum* fat, the proportions in which this acid occurs in the three vegetable oils listed in Table 4 are much lower.

The content of combined hexadecenoic acid in *P. spinulosum* fat is low (3.7%) in comparison with the amounts (15–20%) of this acid in marine-animal oils, but the percentage is high in comparison with the values (usually less than 1% of the total fatty acids) found for this acid in cottonseed oil, peanut oil, soya-bean oil and palmo il. A low percentage of hexadecenoic acid seems to be a characteristic of the fats of a number of moulds which are capable of synthesizing large amounts of

Table 4. Component fatty acids of *Penicillium spinulosum* fat compared with the component fatty acids of fats from other moulds and from vegetable oils

	Free acidity (as oleic acid %)	Iodine value	Saturated acids				Unsaturated acids				
			C ₁₄	C ₁₆	C ₁₈	C ₂₀₋₂₄	C ₁₆ (-2H)	C ₁₈ (-2H)	C ₁₈ (-4H)	C ₁₈ (-6H)	C ₂₀ (-2H)
<i>Penicillium spinulosum</i>	5.8	81.1	Trace	18.0	11.9	1.4	3.8	43.3	21.1	0.3	0.2
<i>Aspergillus nidulans</i>	0.8	73.4	0.7	20.9	15.9	1.4	1.2	40.3	17.0	0.2	2.4
<i>Penicillium lilacinum</i>	0.2	63.7	0.1	32.3	9.4	1.4	3.4	38.6	13.4	Nil	1.4
Palm oil*	Not given	60.0	1.6	32.3	5.5	Nil	Nil	52.4	8.2	Nil	Nil
Olive oil†	Not given	84.2	1.2	15.6	2.0	Nil	1.6	64.6	15.0	Nil	Nil
Groundnut oil‡	Not given	86.5	Nil	8.8	4.5	6.1	Nil	60.3	20.3	Nil	Nil

* Results of Bank *et al.* 1935.

† Results of Gunde & Hilditch, 1940.

‡ Results of Crawford & Hilditch, 1950.

fat from carbohydrate. For example, the figures for hexadecenoic acid in the fats of *A. nidulans* and *P. lilacinum* are respectively 1.2 and 3.4 % (Table 4); likewise the corresponding figures for the fats synthesized by *Penicillium flavo-cinereum* and *Penicillium soppi* are 1.5 and 3.3 % respectively (Singh *et al.* 1956b; Singh *et al.* 1957). Thus the proportions of combined hexadecenoic acid in the fats of these particular moulds are fairly close to those found in mammalian depot fats (2–3 %) and milk fats (3–4 %) (Hilditch, 1956).

In *P. spinulosum* fat the proportion of combined oleic acid, although of much the same order as in the fats of certain other moulds (*A. nidulans*, *P. lilacinum*, *P. flavo-cinereum* and *P. soppi*), is substantially less than in palm oil, olive oil or groundnut oil (Table 4).

Finally, in *P. spinulosum* fat the proportion (21.1 %) of combined linolenic acid lies between the proportions in which it occurs in the fats of *A. nidulans* (17.0 %) and of *A. flavus* (23.7 %) (Singh, 1957). The corresponding value for *P. soppi* fat was found by Singh *et al.* (1957) to be 20.0 %. The lowest and highest values for this component in mould fats examined by Singh *et al.* (1956a, b) are *P. lilacinum* (13.4 %), *P. flavo-cinereum* (27.1 %). Whereas the linoleic acid content of groundnut oil may vary, in different samples, from a value (20 %) very close to that of *P. spinulosum* fat to a much higher figure (35 %), neither palm oil (8.2 %) nor olive oil (15.0 %) incorporates so high a proportion of this unsaturated acid (Table 4). In view of the importance of linoleic acid in nutrition, the fat of *P. spinulosum* should compare favourably, in this particular respect, with these three vegetable oils.

SUMMARY

1. The component acids of fat produced by *Penicillium spinulosum* grown in aerated deep culture have been found to be (%): palmitic, 18.0; stearic, 11.9; arachidic, 1.4; hexadecenoic, 3.8; oleic, 43.4; linoleic, 21.1; linolenic, 0.3; eicosenoic, 0.2.

2. The fat, like the fats of the moulds *Aspergillus nidulans* and *Aspergillus flavus*, has a relatively high stearic acid content in comparison with the fats of other micro-organisms.

3. The hexadecenoic acid content, like the

hexadecenoic acid contents of the fats obtained from a number of other fat-rich mould fungi, is small.

4. The linoleic acid content of the fat is greater than the linoleic acid contents of the more unsaturated olive oils and approaches the proportions normally present in groundnut oils.

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