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Determination of Fatty Acid Composition in Some Pig Fats by Gas Chromatography

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The fatty acid composition in back fat and liver lipids of pigs fed γ-irradiated and non-irradiated potatoes, respectively, have been investigated by gas chromatography using an argon-Pye chromatograph. The best separation of the fatty acid methyl esters was achieved with diethylene glycol succinate polyester as stationary phase. A suitable analysis temperature was found to be 164°C. Diethylene glycol adipate did not give a satisfactory separation. The diet containing γ-irradiated potatoes apparently did not cause any differences of the fatty acid composition in the pig fats as compared with the diet containing control potatoes.

As part of an investigation on the influence of ionizing radiation on potato tubers the aim of the present study was to examine the fatty acid composition in some fats of pigs fed γ-irradiated and non-irradiated potatoes, respectively.

The greater part of the carbohydrates in the diet, which is not used for katabolism, is transformed into fats and is found in the subcutaneous fat layer. Thus, the primary object was to ascertain how far the quantitative proportions of the various component fatty acids in the back fat varied in relation to the feeding on irradiated and non-irradiated potatoes, respectively. In an earlier investigation [1] a somewhat lower percentage of total fat was found in the liver of pigs fed on irradiated potatoes as compared with the control pigs fed on non-irradiated ones. For this reason the second object was to perform some determinations of the fatty acid composition also in the liver lipids.

MATERIALS AND METHODS

Potato tubers of a common Swedish variety were irradiated with γ-rays (14—15 kilorad; dose rates 625 r/h and 175 r/sec). Pigs for breeding were, for about four months, fed the following daily diet: 4 kg cooked potatoes and 2 kg food concentrate, the latter consisting of 68.5 % oats and barley 1:1, 24 % wheat bran, 4 % fish meal, 1 % fodder yeast, 1.5 % chalk, 0.5 % sodium chloride, and 0.5 % vitamins (A + D). One boar and

three sows were fed irradiated potatoes, and one boar and one sow were fed non-irradiated control potatoes of the same potato variety. Fattening pigs, five in each group, the one group given irradiated potatoes and denoted as I-group, the other one given non-irradiated control potatoes and denoted as C-group, were fed from about 25 kg to 90 kg live weight, *i.e.* for 15 weeks. As to the composition of the ration (potatoes:food concentrate), see Ref.[1] During this feeding period more than 200 kg potatoes were consumed by each fattening pig.

Immediately after the slaughter a strip of the back fat was cut out on each side of the spine. Care was taken to get the strips of equal width and until equal depth throughout the entire fat layer, because the composition of the fatty acids varies in different layers of the back fat.[2,3]

The strips were frozen in dry ice and stored frozen (−20°C) until analysing. From the breeding pigs, which consumed about 500 kg potatoes under the period of this feeding experiment, the samples of back fat were cut out the day after slaughter and the strips were frozen in the same way as the strips from the fattening pigs.

From the samples the fat was extracted by grinding, melting, pressing, and filtering.[3] Aliquots of the fat were saponified and converted into the fatty acids according to Hilditch.[4] The fatty acids were subsequently converted to their methyl esters.[4]

The total lipids from the liver (glycerides and phosphatides) were extracted by means of the Schmid-Bondzynski-Ratzlaff method.[5] The saponification and the methylation were performed similarly as for the back fat.

The analyses were carried out in a Pye gas chromatograph, using a column of diethylene glycol succinate polyester (Perkin-Elmer) at 164°C and with argon as carrier gas at a flow rate of 40 ml/min. By using ethylene glycol adipate polyester no satisfactory separation was achieved, whether at 178° nor at 164°C. For analysing and identifying the single components of the fatty acid methyl ester mixture from each pig (back fat and liver) at least four duplicate runs were carried out. For identification of the different peaks, samples of known fatty acid methyl esters were injected one by one together with the mixture to be separated and investigated, and runs were performed with and without these additional test substances.

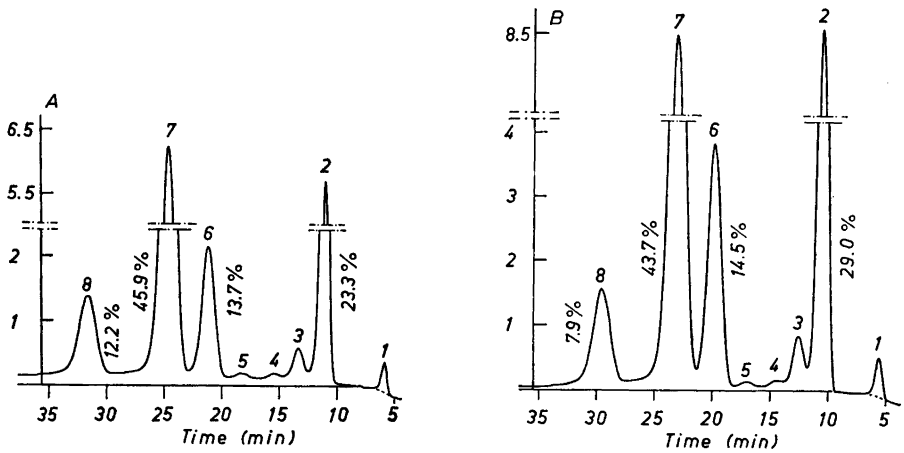

Fig. 1. Gas-liquid chromatograms of fatty acids (as methyl esters) from back fat of pigs. A: pig from C-group; B: pig from I-group. 1 = myristic; 2 = palmitic; 3 = palmitoleic; 4 = isomeric palmitoleic or heptadecanoic?; 5 = palmitolenic or heptadecenoic?; 6 = stearic; 7 = oleic; 8 = linoleic.

RESULTS

Diagrams for two typical runs are shown in Fig. 1. It can be seen from this figure that there are only minor differences in the composition of the fatty acids in back fat derived from pigs in the different groups. The major components found are: palmitic, stearic, oleic, and linoleic acids. The content of linoleic acid found is, however, somewhat higher in back fat from pigs in the C-group than from pigs in the I-group. The mean values of the different fatty acids for all pigs in each group are recorded in Table 1. The fourth column of this table shows the percentage of fatty acids in back fat from pig No. 9 in the C-group. This pig diverged from all the other fattening pigs in having an extremely high content of linoleic acid. From determinations in an earlier investigation [1] it appeared that the iodine value for this same fat was extremely high, the "keepability" however very low. The seventh column in Table 1 shows the values for fatty acids investigated in a sample mixed of back fats from 100 different sows. The values found are in good accordance with those reported by the supplier of this sample.[5]

Table 1. Fatty acid composition of back fat from pigs fed irradiated (I-group) and non-irradiated potatoes (C-group). The figures are mean values for all pigs in the same group.

Fatty acid	Fatty acids as percentage of total fatty acids					
	Fattening pigs			Breeding pigs		Mixed sample
	I-group	C-group	Pig No. 9	I-group	C-group	
Saturated:						
Myristic	1.4	1.4	1.2	1.3	1.2	1.3
Palmitic	26.6	25.2	22.9	23.8	21.9	23.5
Heptadecanoic [a]	0.8	0.7	0.6	0.5	0.5	0.9
Stearic	15.4	13.9	12.7	10.4	12.5	10.9
Unsaturated:						
Palmitoleic	2.8	2.8	2.6	3.2	2.8	3.3
Heptadecenoic [b]	0.7	0.7	0.8	0.3	0.4	0.2
Oleic	44.0	44.1	46.4	49.7	47.7	50.2
Linoleic	7.9	9.5	12.7	9.9	10.8	9.8
Poly-unsaturated $C_{20}-C_{22}$	Trace	Trace	Trace	Trace	Trace	Trace

[a] Not identified; may be isomeric $C_{16:1}$

[b] Not identified; may be $C_{16:2}$; *cf.* Privett *et al.*[8]

The result of the present investigation is that no important differences have been observed in the fatty acid composition in back fat from pigs fed irradiated potatoes compared with pigs fed non-irradiated control potatoes. The content of the fatty acids found in the back fat are, in order of magnitude: oleic, palmitic, stearic, linoleic acid, and minor amounts of palmitoleic, and myristic acid, which result is in good agreement with reports of other investigators.[6,7]

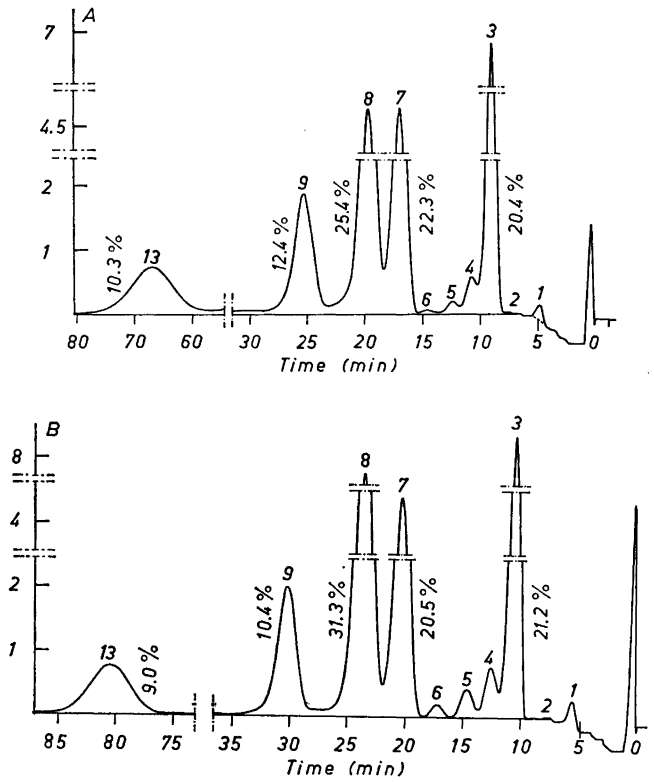

Fig. 2. Gas-liquid chromatograms of fatty acids (as methyl esters) from pig liver lipids. A: pig from C-group; B: pig from I-group. 1 = myristic; 2 = unidentified; 3 = palmitic; 4 = palmitoleic; 5 = see 4 in Fig. 1; 6 = see 5 in Fig. 1; 7 = stearic; 8 = oleic; 9 = linoleic; 13 = "arachidonic".

Two additional components were detected with R_F values between those for palmitoleic and stearic acid. These are perhaps saturated and unsaturated C_{17} acids. Odd number fatty acids in minor amounts have been found in pork fats, *e.g.* by Privett *et al.*[8]

Regarding the liver lipids the same main saturated and unsaturated fatty acids were found as in the back fat. The proportions between the components differed in some points from those examined in the back fat, *e.g.* higher content of stearic acid and lower content of oleic acid has been found in the liver lipids. The gas chromatogram (see Fig. 2) also shows a peak at a R_P (P = palmitic acid) value of 6.5. This obviously represents arachidonic acid and some C_{22} acids (the sum called "arachidonic acid" below) reported by other investigators to be present in liver lipids,[8-11] not identified in this study, however.

Table 2 illustrates earlier and recent reports on the composition of fatty acids in liver lipids as compared with the present investigation. As can be seen

from the table the sum of the unsaturated acids found in the present study is almost the same as the sum reported by Privett *et al.*[8], somewhat lower, however, than the corresponding value taken from the earlier investigation by Hilditch and Shorland.[10] The content of oleic acid found was lower, the contents of linoleic and "arachidonic" acids, however, higher as reported by Privett *et al.*[8] Also minor components of higher fatty acids with R_F values between linoleic and "arachidonic" acid, in all about 1 %, were observed.

Table 2. Fatty acid composition of pig liver lipids according to earlier and recent reports as compared with the present investigation.

Fatty acid	Hilditch *et al.*[10] Fatty acid %	Privett *et al.*[8] Calculated Fatty acid %	Gas chromatographically derived values in the present investigation	
			Fatty acid %	
			I-group	C-group
Saturated:				
Myristic			0.4	0.5
Palmitic	20.6	22.9	18.2	17.9
Stearic	18.3	16.8	20.7	22.3
Arachidic	2.0			
Total	40.9	39.7	39.3	40.7
Unsaturated:				
Palmitoleic	0.5	0.6	1.6	1.8
Isomeric palmitoleic		0.6	2.0[a]	1.4[a]
Palmitolinoleic		0.2	0.5[b]	0.5[b]
Oleic	25.1	38.5	29.3	25.4
Linoleic		5.0	10.4	12.6
Isomeric linoleic		0.2		
Octadecatrienoic		0.3		
Eicosadienoic		0.2		
Eicosatrienoic	31.6	0.3		
Arachidonic		4.4		
Isomeric arachidonic		0.2		
Eicosapentaenoic		0.2	9.0	11.9
Docosadienoic		0.4		
Docosapentaenoic	1.9	0.2		
Docosahexaenoic		0.3		
Total	59.1	51.5	52.8	53.6

[a] Not identified; may be C_{17}.
[b] Not identified; may be $C_{17:1}$; *cf.* Table 1.

DISCUSSION

The proof that carbohydrates must be an important source of animal fat was first rigidly given by Lawes and Gilbert[12] in 1860—1866. A full examination was made by Hilditch *et al.*[6] of the component acids in the deposited fats

of pigs reared on known diets. Nunn and Smedley-MacLean [13] found that rats on a fat-free diet yielded liver fats without tetraenoic or pentaenoic acids, but that on the same diet with the addition of a little methyl linoleate, the liver fats contained some arachidonic acid; whilst with the addition of methyl linolenate, both arachidonic and docosapentaenoic acids appeared in the liver lipids. Klenk and Oette [9] reported similar results; in addition they found eicosapentaenoic and docosahexaenoic acid in liver phosphatides when the rats were fed linolenic acid. By feeding fish meal to pigs Banks and Hilditch [14] found highly unsaturated C_{20} and C_{22} fatty acids deposited in the fat. Hilditch *et al.*[6] have shown that no synthesis of linoleic acid seems to occur in the pig organism.

As the pigs in the present investigation, besides potatoes, received in the food concentrate oats, fish meal, and A + D vitamins, and since the fat content in potato tubers is nearly negligible, the linoleic acid and the other higher unsaturated fatty acids found in the back fat and liver lipids evidently does not origin from the potato diet. Thus, the small differences between linoleic acid contents in back fat from pigs in the I-group and the C-group, found in the present investigation, are clearly not caused by ingestion of potatoes, neither irradiated nor control ones. It should be pointed out that the pigs, the whole group of five reared in the same box, had the opportunity to consume more or less of the different food components. This fact may be the explanation of the exceptional values found in the back fat from the control pig No. 9. On the other hand, potato tubers are a very complex system from the chemical point of view, containing for instance about thirty different enzymes. The mechanism disturbed in the potato tubers by γ-irradiating is not yet cleared up. It is only known that the germination is irreversibly inhibited, and chemical and enzymatic changes,[15–17] more or less reversible, have been observed. Thus anyone of these changes could perhaps also have influenced the fatty acid composition in the fats of the pigs. It has been demonstrated by Swaminathan *et al.*[18,19] that the root meristems derived from barley embryos, grown on mash from irradiated potato tubers differ from such cultivated on mash from control tubers. They report a eight-fold increase in the frequency of occurrence of cells with micronuclei as a result of irradiation. Special care should therefore be exerted in examinations of changes in all systems investigated. Even minor alterations, caused by irradiation, should be taken in consideration since they can, in course of time, cause important effects in the organism.

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REFERENCES

1. Jaarma, M. and Henricson, B. *Acta Vet. Scand.* **5** (1964). *In press.*
2. Dean, H. K. and Hilditch, T. P. *Biochem. J.* **27** (1933) 1950.
3. Dahl, O. *Acta Agr. Scand.* **8** (1958) 106.

4. Hilditch, T. P. *The Chemical Constitution of Natural Fats*, Chapman & Hall, London 1956, pp. 572, 575.
5. Dahl, O. *Personal communication.*
6. Hilditch, T. P., Lea, C. H. and Pedelty, W. H. *Biochem. J.* **33** (1939) 493.
7. Youngs, C. G. *J. Am. Oil Chemists' Soc.* **36** (1959) 664.
8. Privett, O. S., Weber, R. P. and Nickell, E. C. *J. Am. Oil Chemists' Soc.* **36** (1959) 443.
9. Klenk, E. and Oette, K. *Z. physiol. Chem.* **318** (1960) 86.
10. Hilditch, T. P. and Shorland, F. B. *Biochem. J.* **31** (1937) 1499.
11. Kirschman, J. C. and Coniglio, J. G. *Arch. Biochem. Biophys.* **93** (1961) 297.
12. Lawes, J. B. and Gilbert, J. H. *J. Roy. Agr. Soc. Engl.* **21** (1860) 433.
13. Nunn, L. C. A. and Smedley-MacLean, J. *Biochem. J.* **32** (1938) 2178.
14. Banks, A. and Hilditch, T. P. *Biochem. J.* **26** (1932) 298.
15. Jaarma, M. *Arkiv Kemi* **13** (1958) 97.
16. Jaarma, M. *To be published.*
17. Schwimmer, S., Burr, H. K., Harrington, W. O. and Weston, W. J. *Am. Potato J.* **34** (1957) 31.
18. Swaminathan, M. S., Chopra, V. L. and Bhaskaran, S. *Radiation Res.* **16** (1962) 182.
19. Chopra, V. L. and Swaminathan, M. S. *Naturwiss.* **50** (1963) 374.

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