IDENTIFICATION OF ANIMAL FAT SPECIES

R. VERBEKE and H. DE BRABANDER

Laboratory of Chemical Analysis of Food of Animal Origin, Veterinary Faculty of the University of Ghent, Belgium

INTRODUCTION

ESTIMATION of animal fat adulteration is also of interest when the origin of meat species present in meat products is to be determined. This problem is frequently met if, through curing and processing, the meat proteins are denaturated rendering species specific protein detection with serology or electrophoresis techniques impossible. The only valuable approach left is to determine species specific heat stable components in the meat products.

Chicken meat in heated pork has been detected by the carnosine/anserine ratio (12). However, admixture of beef or other species decreases the sensitivity of the method. Characterisation of animal fats, based on typical fatty acid ratios has been repeatedly reported (3,4). Since feeding regime may significantly affect the fatty acid composition, this discrimination of species on basis of fatty acid analysis is of doubtful value. The high affinity of palmitic acid to position 2 in pork fat triglycerides has been reported to be species specific (2,7). Recently we found that beef and pork fat are characterized by different but close correlationships between the incorporation of certain fatty acids into the 2-position and the corresponding content of fatty acids in the total triglycerides (13). Since that time we extended these observations and studied the inter-relationships between different fatty acids within the triglycerides of pork, beef, horse and chicken. It is suggested that some relationships are species specific and can be used as a reliable method in the determination of fat mixtures.

MATERIALS AND METHODS

IN AN EARLIER paper (13) the beef and pig fats analysed were described. In addition 11 samples of pig fat (including 6 "unsaturated" pig samples (8)), 14 samples of subcutaneous and kidney fat of horses (Poland, U.S.A., Bristol, Sussex, Argentina) and 24 samples of fats isolated from breast, around stomach and thigh fat of hens (HYBRO, Warrens sexselinnen, Hubbard and 1 Turkey) were analysed. In the hen series, fat was also extracted from the lean meat of the thigh. The triglycerides were extracted from meat in chloroform-methanol and isolated by TLC-chromatography (silagel 60). The fat tissue samples were homogenised, melted and filtered at 80 °C. The clear fat was stored in the freezer (-20 °C) until used.

Fats were transesterified by incubating 20 mg fat in presence of 1 ml sodium methylate solution (0.025 N) in methanol at 90 °C during 1 h. The fatty acid composition in position 2 of the triglycerides was determined by a modification of the method described before (13). Pancreatic lipase (100 mg ; E.C.n° 3.1.1.3 ; Sigma type II) was homogenised with 1 ml 1 M TRIS-buffer (pH = 8.2). On a piece of ground glass of 1.5 x 7 cm (e.g. a cover of a tank) 250 µl lipase solution was applied. A homogeneous lipase reaction band was formed on silicagel plates (10 x 20 cm) by gently pushing the plate against the ground glass piece. 100 µl of a fat solution (80 mg of fat in 1 ml n-hexane) was evenly applied over the lipase reaction band. The silicagel plate was placed immediately in a waterbath (40 °C) with the silicagel layer situated at 2 cm above the water surface. After 10 min. incubation the plate was removed and dried carefully. The lipid mixture was concentrated into a narrow band by developping the plate three times with diethylether-formic acid (98:2, v/v) over a distance of ± 5 cm. The lipase reaction band was removed by cutting off that part of the plate. The remainder of the plate was developped in n-hexane-diethylether-formic acid (80:20:2, v/v/v). After drying, the monoglyceride fraction was transferred into a small column (0.6 mm I.D.) and elution was performed with 2, 1 and 1 ml freshly distilled, dry diethylether. The ether was evaporated under a jet of nitrogen. The lipids were transesterified with 200 µl sodium methylate solution. The gaschromatograph used was a Varian 3700. A capillary column (50 m ; 0.25 mm I.D. ; R.S.L. ; Belgium) coated with Silar 10 C was used. The carriergas was H_2 at 2 ml/min. The temperature of the column, the injector and the detector was at 160, 210 and 220 °C respectively.

RESULTS AND DISCUSSION

1. Variations in fatty acid distribution among the triglycerides

THE FATTY acid analysis of pig, beef, horse and hen fats are shown in table 1. The large variations observed in the fatty acid contents of the triglycerides do not allow discrimination of the animal species on basis of its fatty acid percentages. However, stereospecific analysis of the fats has shown that animal fats may be qualitatively identified by characteristic asymmetric distribution of their fatty acid constituents (2). Pancreatic lipase hydrolyses specifically the ester bonds at the 1,3-positions of the triglycerides (9). Analysis of the monoglycerides formed during pancreatic hydrolysis allows determination of the

Table 1 : Mean fatty acid composition (mole %) of whole triglycerides and of fatty acids at the 2- and 1,3-positions of pig fat, beef tallow, horse fat and hen fat.

Fatty acid	Mean fatty acid content ± standard error					
	C16:0	C16:1	C18:0	C18:1	C18:2	C18:3
Pig fat (n = 21)						
in triglyceride	26.6 ± 3.6	2.3 ± 0.6	12.3 ± 3.1	40.5 ± 5.4	13.1 ± 8.9	1.7 ± 0.7
in 2-position	65.8 ± 5.7	3.3 ± 0.7	4.2 ± 0.6	12.1 ± 1.9	5.4 ± 5.4	1.3 ± 1.0
in 1,3-position	6.9 ± 2.8	1.7 ± 0.5	16.4 ± 4.5	54.8 ± 7.8	17 ± 11	2.5 ± 1.1
proportion in 2-position	83 ± 6	49 ± 6	12 ± 2	10 ± 2	12 ± 3	21 ± 11
Beef tallow (n = 10)						
in triglyceride	28.9 ± 2.6	2.2 ± 0.6	29.6 ± 4.8	25.5 ± 3.8	1.4 ± 0.6	
in 2-position	18.2 ± 2.3	3.7 ± 1.0	12.3 ± 2.5	40.2 ± 5.6	2.5 ± 1.0	
in 1,3-position	34.2 ± 4.1	1.5 ± 0.6	38.2 ± 6.2	18.1 ± 3.4	0.9 ± 0.6	
proportion in 2-position	21 ± 3	58 ± 11	14 ± 2	53 ± 4	62 ± 18	
Horse fat (n = 14)						
in triglyceride	33.0 ± 2.3	8.0 ± 2.7	3.8 ± 0.9	30.2 ± 2.6	5.9 ± 1.4	9.7 ± 5.5
in 2-position	12.1 ± 2.7	12.5 ± 4.4	2.9 ± 0.9	33.9 ± 3.4	11.2 ± 3.0	12.8 ± 8.6
in 1,3-position	43.4 ± 4.1	5.7 ± 2.2	4.3 ± 1.2	28.7 ± 2.2	3.3 ± 1.3	8.1 ± 4.6
proportion in 2-position	12 ± 3	52 ± 6	26 ± 8	37 ± 2	64 ± 10	43 ± 10
Hen fat (n = 24)						
in triglyceride	27.1 ± 4.1	5.4 ± 1.8	6.3 ± 1.6	41.4 ± 4.8	17.3 ± 4.8	1.0 ± 0.3
in 2-position	19.6 ± 7.7	3.6 ± 1.3	7.4 ± 3.0	45.3 ± 9.2	21.8 ± 6.6	0.8 ± 0.3
in 1,3-position	30.8 ± 3.1	6.3 ± 2.1	5.7 ± 2.6	39.5 ± 4.3	15.0 ± 4.1	1.1 ± 0.4
proportion in 2-position	23 ± 6	23 ± 5	41 ± 18	36 ± 5	42 ± 4	27 ± 5

fatty acids esterified at the 2-position of glycerol. From table 1, it is evident that the relative distribution of the fatty acids over the 2-position and 1,3-positions of the triglycerides can be used to identify qualitatively the fats studied.

Pig fat is exceptional by its efficient incorporation of palmitic acid at the 2-position of glycerol (7,10), while the unsaturated fatty acids are preferentially esterified at the 1,3-position of the triglycerides. The proportion of oleic acid incorporated at position 2 of horse and hen triglycerides differs significally from fats of pig and beef (table 1). Stearic acid is selectively incorporated into the 1,3-positions of beef fat if compared to the results on horse or hen fat. Chicken fat is characterized from other fats by its low proportion of palmitoleic acid incorporated at the 2-position of the triglycerides. The total linolenic acid (C18:3) content of horse fat showed large variations and cannot be used as a reliable parameter identifying this fat. However, within the species studied, large variations are found in the positional distribution of the fatty acids among the fats. This may be expected since the fatty acid spectrum and the relative distribution of the fatty acids within the triglycerides may depend on feeding regime (5,8), additives (1) and anatomical location of the fat (5,7).

2. Fatty acid correlations within the triglycerides

RECENTLY, we demonstrated that adulteration of pig fat with beef fat can be estimated on basis of regression equations (13). In agreement with our earlier results, it is found that in pig fat palmitic acid esterified at position 2 is closely correlated with its content at position 1,3 of the triglycerides (fig. 1). A different, but significant regression is found between the palmitic acid contents of position 2 and position 1,3 of hen fat triglycerides.

As shown in fig. 2, close but different correlations exist between the amounts of oleic acid + linoleic acid (C18:(1+2)) incorporated in position 2 and its corresponding content in the total triglycerides of the different species studied. Linear relationships are found between the concentration of stearic acid in position 2 and its contents in total triglycerides in pig ($r = 0.59$, $p \leq 0.01$), beef ($r = 0.84$, $p \leq 0.01$) and horse ($r = 0.40$) fats. Different proportionality equations are observed between the molar percentages of palmitoleic acid in position 2 and the triglycerides of horse fat ($r = 0.66$, $p \leq 0.01$), chicken fat ($r = 0.72$, $p \leq 0.001$) and pig fat ($r = 0.80$, $p \leq 0.001$). Moreover it is found that the molar percentages of some fatty acids are closely interrelated within the the positions of the triglycerides. At position 2 of the triglycerides, stearic acid has been found to increase with the palmitic acid content in fats of pig ($r = 0.49$, $p \leq 0.05$), beef ($r = 0.82$, $p \leq 0.01$), horse ($r = 0.84$, $p \leq 0.001$) and chicken ($r = 0.91$, $p \leq 0.001$). The negative correlations between stearic acid and the unsaturated fatty acids incorporated at the 1,3-position of the triglycerides of pig ($r = -0.94$, $p \leq 0.001$) and beef ($r = -0.77$, $p \leq 0.01$) may explain the inverse relationships between stearic acid and oleic acid observed in pig (1) and ruminant (5) depot fats. Since we included in our study fats from

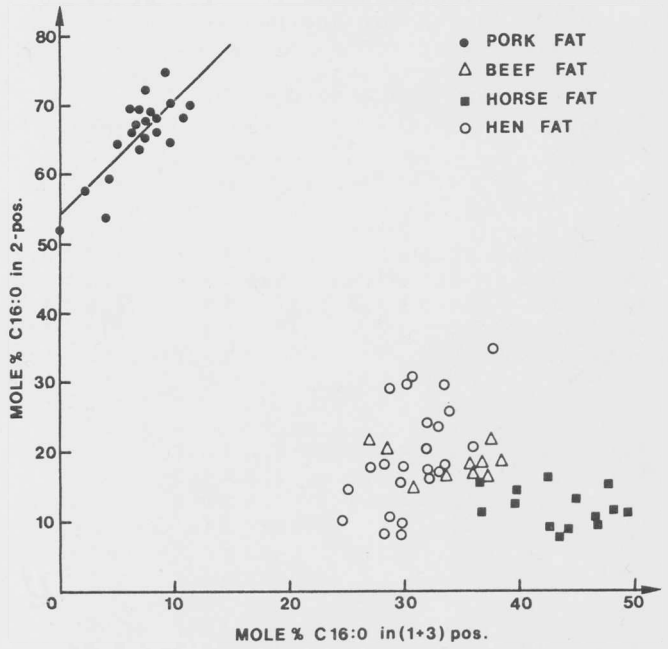

Fig. 1 : Relationship between incorporation of palmitic acid in the 2-position (= Y) and its content in the (1+3)-position (= X)

Pork fat : $Y = 1.66\ X + 54.3$ $\quad r = 0.79^{a}$
Hen fat : $Y = 1.37\ X - 22.8$ $\quad r = 0.56^{b}$

^{a} $p < 0.001$; ^{b} $p < 0.01$

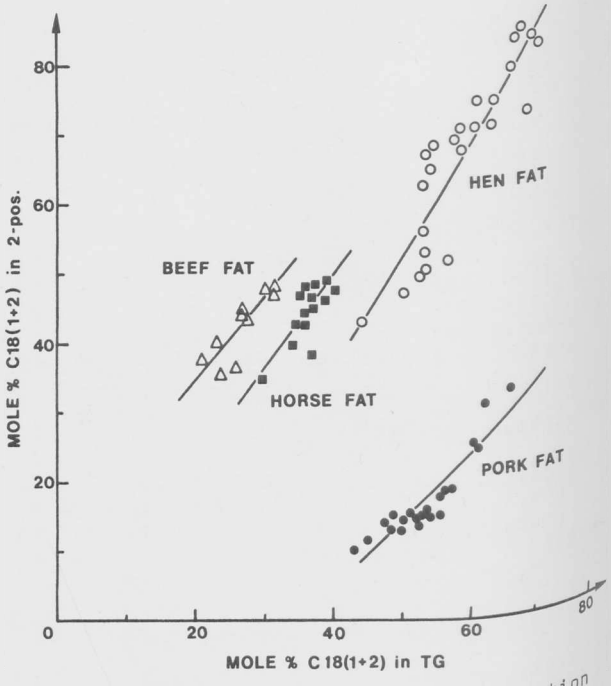

Fig. 2 : Relationship between incorporation of oleic + linoleic acid in the 2-position (= Y) and corresponding contents in the triglycerides (= X)

Pork fat : $Y = 0.96\ X - 34.0$ $\quad r = 0.92^{a}$
Hen fat : $Y = 1.60\ X - 27.2$ $\quad r = 0.89^{a}$
Horse fat : $Y = 1.34\ X - 4.0$ $\quad r = 0.82^{a}$
Beef fat : $Y = 1.21\ X + 10.4$ $\quad r = 0.87^{a}$

^{a} $p < 0.001$

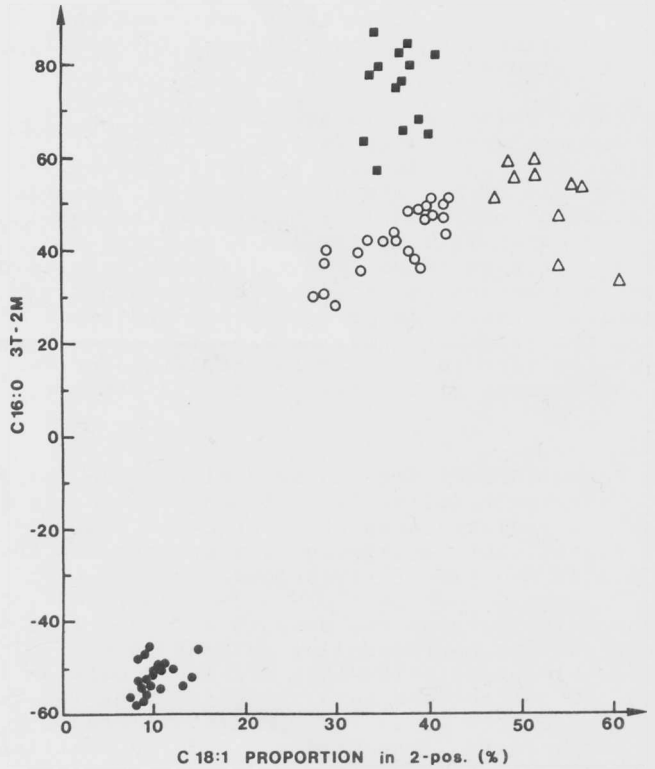

Fig. 3 : Discrimination of pork fat (•) from horse (■), beef (Δ) and hen fat (o)

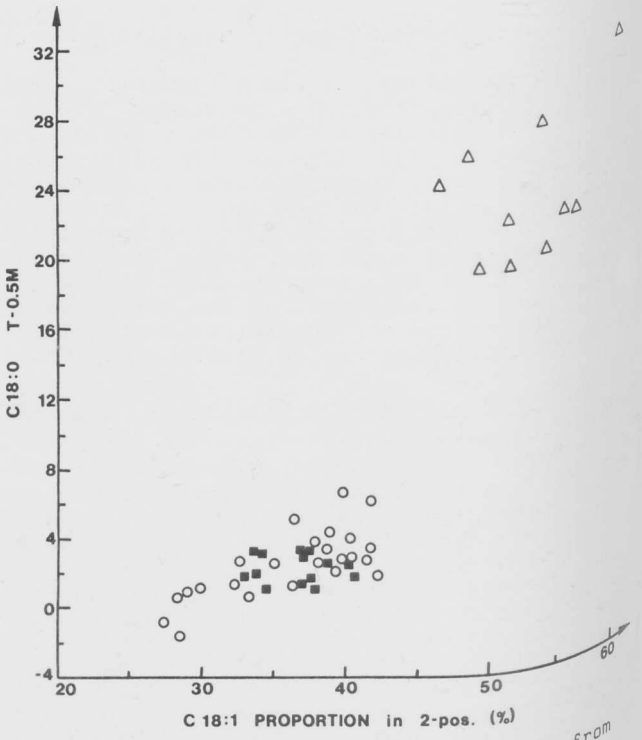

Fig. 4 : Discrimination of beef fat (Δ) from horse (■) and hen fat (o)

$$\text{Proportion in 2-position \%} = \frac{\text{Mole \% in 2-pos.}}{3 \times \text{Mole \% in TG}} \times 100$$

T = Mole % of fatty acid in triglyceride
M = Mole % of fatty acid in 2-position

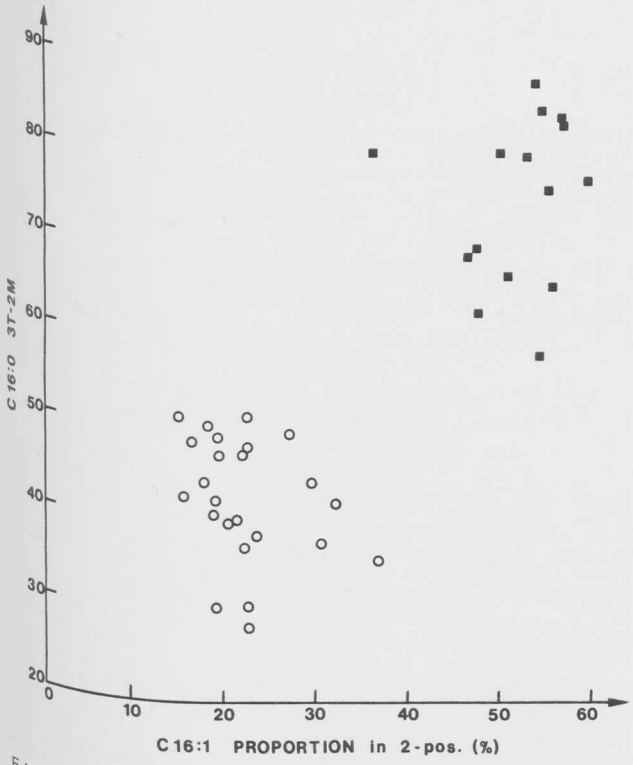

Fig. 5 : Discrimination of horse fat (■) from hen fat (o)

animals on different feeding regime, breed and anatomical location the results suggest that some of the correlations may be typical for the species studied.

3. Quantitative determination of fats in mixtures

PIG fats are effectively discriminated from other fats by the positional distribution of palmitic acid and unsaturated fatty acids within the triglycerides. This is illustrated in fig. 3 in which the proportion of oleic acid in position 2 is plotted against a linear combination of the palmitic acid content of the 2-position (M) and total triglyceride (T). Using 95% confidence limits it was calculated that addition of 10% either beef, horse or chicken fat can be detected in pig fat with a propability of 84%. These results indicate that this technique is at least as sensitive as the Bömer-method in detecting adulteration of pig fat with beef tallow (11). Moreover, the proposed method allows a reliable and quantitative estimation of different fats added to pig fat. In contrast, addition of 20% pig fat to either hen, beef or horse fat can be determined with a propability of 84% and using 95% fiducial limits.

Beef fat can be discriminated from horse or hen fats on basis of its oleic acid proportion in position 2 and the distribution of stearic acid within the triglycerides (fig.4). Use of these parameters allow estimation of either 15% horse or hen fat in beef fat. Accepting 95% confidence limits, 30% horse or chicken fat can be detected in beef fat with a propability of 84%.

Horse fats and chicken fats show large differences in the proportions of palmitoleic acid in position 2 and in the distribution of palmitic acid within the triglycerides (fig. 5). Addition of 20% chicken fat to horse fats can be determined. Due to the relatively large variations observed in the chicken fat measurements, only 40% of chicken fat can be estimated in horse fat with a propability of 84% at the 95% confidence interval.

Through fat analysis, the relative amounts of pork meat in sausages containing beef, horse or chicken meat can be estimated. From the total fat content and after pancreatic lipase analysis of the triglycerides isolated from meat, the relative percentages of pig fat in other fats was determined. Assuming a typical fat percentage for one of the meat constituents, the relative composition of the meat product is calculated. Using this procedure the amount of chicken meat in pork or beef sausages was calculated with a reasonable degree of accuracy.

REFERENCES

1. AMER, M.A. and ELLIOT, J.I. (1973) Can. J. Anim. Sci. 53, 147-152
2. BROCKERHOFF, H., HOYLE, R.J. and WOLMARK, N. (1966) Biochim. Biophys. Acta 116, 67-72
3. CARISANO, A. and RIVA, M. (1976) Riv. Ital. Sost. Grasse 53, 297-300
4. DARO, B. (1977) Riv. Ital. Sost. Grasse 54, 349
5. GARCIA, P.T., CASAL, J.J., PARODI, J.J. and MARANGUNICH, L. (1979) Meat Science 3, 169-177
6. GARTON, G.A. and DUNCAN, W.R.H. (1965) Biochim. Biophys. Acta 98, 436-438
7. HILDITCH, T.P. and WILLIAMS, P.N. (1964) in "The chemical constitution of natural fats" p. 409, 4 th Ed., Chapman and Hall, London
8. HOUBEN, J.H. and KROL, B. (1978) XXIV th European Meeting of Meat Research Workers, Kulmbach, paper J. 4
9. MATTSON, F.H. and VOLPENHEIN, R.A. (1961) J. Lipid Res. 2, 58-62
10. MATTSON, F.H., VOLPENHEIN, R.A. and LUTTON, E.S. (1964) J. Lipid Res. 5, 363-365
11. ROOS, J.B. (1962) Fette, Seifen, Anstrichm. 64, 6-12
12. TINBERGEN, B.J. and SLUMP, P. (1976) Z. Lebensm. Unters. Forsch. 161, 7-11
13. VERBEKE, R. and DE BRABANDER, H. (1979) XXV th European Meeting of Meat Research Workers, Budapest, 2, 767-772