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DIFFERENTIATION OF MEAT SPECIES IN PROCESSED MEAT PRODUCTS THROUGH IDENTIFICATION OF ANIMAL FAT SPECIES

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ABSTRACT

The distribution pattern of the major fatty acids over the triglycerides of pork, beef, horse and chicken fat was shown to be species specific and was used for the discrimination of fat species. Through analysis of the fatty acid composition of the triglycerides and the monoglycerides, isolated after a lipase treatment of the fats, the relative percentage of the fat species in the fat mixture can be reliably determined. Assuming typical fat percentages of the meat species the relative amounts of pork, beef, horse and chicken meat in the product can be estimated.

INTRODUCTION

There is a need for simple and reliable analytical methods to determine the animal species from which processed meat products are made. Curing, processing and intensive meat treatment results in a denaturation of sarcoplasmic proteins hampering species specific detection by serological or electrophoretic methods. The only valuable approach in identifying the meat species in heat processed products should be based on a determination of the species specific heat stable components in the meat products.

Chicken meat in heated pork has been detected by the anserine/carnosine ratio (Olsman and Slump, 1981). However, admixture of beef or other species decreases the sensitivity of the method. Characterisation of animal fats by fatty-acid analysis, using "typical" fatty acid ratios, has been repeatedly reported (Carisano and Riva, 1976; Doro, 1977). However, since the feeding regime may significantly affect the fatty acid composition, the discrimination of species on basis of fatty acid analysis is of doubtful value.

It has since long been known that pork fat is exceptional in that position 2 is largely occupied by palmitic acid (Hilditch and Williams, 1964). Adulteration of beef fat with pork fat has been detected on basis of the proportion of palmitic acid at the 2-position (Verbeke and De Brabander, 1979a; El Sayed and Dashlauty, 1979) or the appearance of pig fat triglycerides during argentation chromatography (Barrett et al., 1963). Using lipase analysis we observed that fats of pork, beef, horse or hen

were characterized by different but close relationships between the fatty acid concentrations at the 2- or combined 1,3 positions and its corresponding fatty acid content in the total triglycerides (Verbeke and De Brabander, 1979b; 1980). It is shown here that lipase analysis of fats provides a rapid and reliable means in identifying the fat species. In using the proposed procedure, the relative percentage of the fat species in binary fat mixtures can be determined. Since meat is always associated with fat, lipase analysis of this heat stable component allows a rapid species identification of the heat treated meats.

EXPERIMENTAL

Materials and methods

In an earlier paper (Verbeke and De Brabander, 1980) the pig, beef, horse and hen fats analyzed were described. The fats were extracted from meat samples in chloroform-methanol and the triglycerides isolated by TLC-chromatography (silicagel 60). The fat tissue samples were homogenized, 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 (Verbeke and De Brabander, 1979b). 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.

Data analysis

A fat population may be represented as a two-dimensional plot of two variables represented by 'x' and 'y'. These variables are calculated from the fatty acid composition (mole %) of the whole triglycerides and of the fatty acid contents at the 2- and 1,3 positions of the different fats (see Table 1).

The equation of the ellipse, enclosing a number of x, y points is written as (Defrise-Gussenhoven, 1952) :

$$\lambda^2 = \frac{1}{1 - r^2} \left(\frac{(x - \bar{x})^2}{s_x^2} - 2\, r \frac{(x - \bar{x})(y - \bar{y})}{s_x\, s_y} + \frac{(y - \bar{y})^2}{s_y^2} \right)$$

with $\bar{x}$, $\bar{y}$: the mean values of the parameters x and y

s_x, s_y : the standard deviation

r : the coefficient of correlation

λ^2 : constant, which determines the number of samples, enclosed by the ellipse (95 % of the samples enclosed by the ellipse if $\lambda^2 = 5.99$; for 99 % then $\lambda^2 = 9.21$)

From our data, varying mixtures of two fat populations were calculated using a FORTRAN IV computer program VET (e.g. : 21 pig fats x 24 hen fats = 504 mixtures at 20 mixing ratios). For each mixture and for each mixing ratio λ^2 was calculated from the mixing data (x and y) and a number of "so called" discriminant parameters ($\bar{x}$, $\bar{y}$, s_x, s_y and r). The discriminant parameters were selected from previous experiments and from the correlation matrices, calculated from the parameters of the population. The discriminant parameters were tested by calculating the detectionscore in function of the mixing ratio. The detectionscore is defined as the percentage of fat mixtures which are detected as adulterated. Thus at the 5 % significance level λ^2 should exceed the value 5.99; for $p \leq 0.01$ then

$\lambda^2 \geqslant 9.21$.

RESULTS AND DISCUSSION

Distribution pattern of fatty acids in some animal triglycerides

The mean fatty acid composition of the total triglycerides and the 2-monoglycerides obtained by pancreas lipase on pig, beef, horse and hen fats are shown in Table 1. The large variations observed in the fatty acid contents of the triglycerides rule out discrimination of animal species on basis of its fatty acid composition. The variability of the linoleinic acid content excludes an unequivocal identification of horse fat on basis of this parameter.

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±4.6	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

However, stereospecific analysis has shown that some animal fats may be qualitatively identified by characteristic assymmetric distribution of their fatty acid constituents over the triglyceride. In most mammals saturated fatty acids predominate in position 1, shorter chain and unsaturated

fatty acids are found largely in position 2 and stearic acid and longer chain fatty acids are located in position 3 (Brockerhoff, 1966). In contrast, pig adipose triglycerides have an unusual fatty acid distribution in that position 2 is mainly esterified with palmitic acid; the unsaturated fatty acids are concentrated at position 3 while the remaining saturated fatty acids are located at position 1 (Brockerhoff et al., 1966; Christie and Moore, 1970). The results in our pancreatic lipase studies (Table 1; Verbeke and De Brabander, 1980) of different fats agree with the data reported earlier in the literature (Hilditch and Williams, 1964; Mattson et al., 1964; Brockerhoff, 1966; Christie and Moore, 1970). Although we selected fats of widely differing fatty acid composition our results suggest that the relative distribution of the fatty acids over the 2- and the 1,3-position of the triglycerides can be used to identify qualitatively the fats studied. Comparing the variation in the concentration of each individual fatty acid, in each of the 2- or 1,3-positions, with the variation in the concentration of that acid in the entire triglycerides indicates that the variance could be reduced significantly if the results were expressed as the proportion of each fatty acid at all positions (Table 1). This suggests that the concentration of some fatty acids at position 2 or the combined 1,3-positions are proportional to the same fatty acids in the total triglyceride.

Christie and Moore (1970) in studying pig fats of widely differing fatty acid composition, could demonstrate a good correlation between the fatty acid composition of the total triglycerides and the positional distribution of the major acids ($C_{16:0}$, $C_{18:0}$, $C_{18:1}$, $C_{18:2}$, $C_{16:1}$ and $C_{14:0}$) within the triglyceride molecules. We observed that in pig, beef, horse or chicken most fatty acids at position 2 or the combined 1,3-position showed a close correlation to their corresponding contents in the total triglycerides (Verbeke and De Brabander, 1980). The linear correlation coefficients were highly significant ($p \leq 0.01$) and where poor correlation coefficients were obtained it implied that the fatty acid content at the position 2 or 1,3 was constant over the measured ranges of the same fatty acid in the triglyceride. However the different fat species were characterised by significant different regression lines.

As shown in Fig. 1, the incorporation of oleic + linolic acid in position 2 of the triglycerides is linearly related to its oleic + linolic acid content in the triglycerides. For these acids the different fats are characterized by differences in elevation of the regression line. In pig and

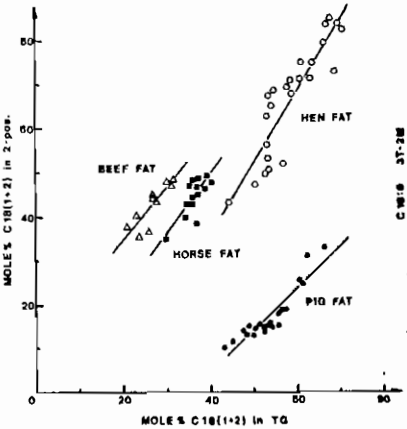

Fig. 1 Relationship between the incorporation of C18(1+2) in the 2-position and corresponding contents in the triglycerides.

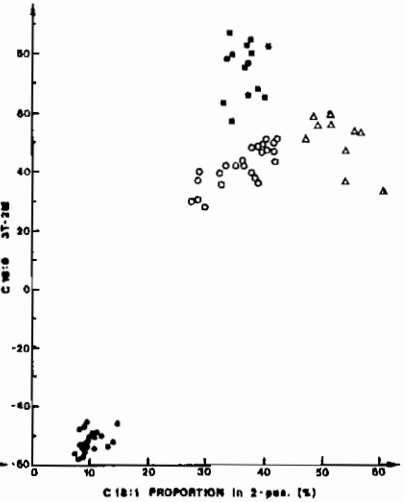

Fig. 2 Discrimination of pork fat (●) from horse (■) beef (▲) and hen fat (○).

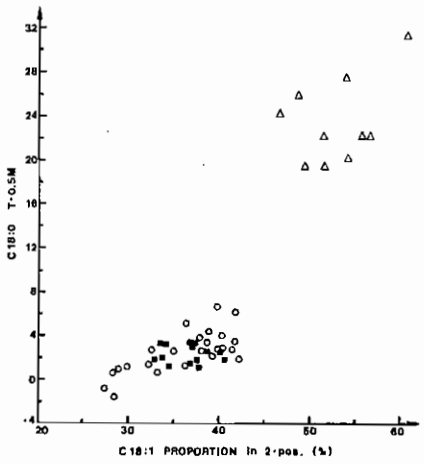

Fig. 3 Discrimination of beef fat (▲) from horse (■) and hen fat (○).

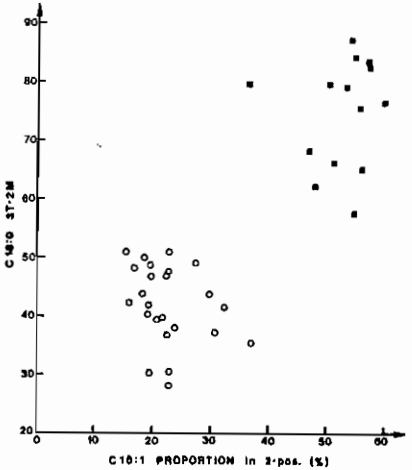

Fig. 4 Discrimination of horse fat (■) from hen fat (○).

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

hen fat, the palmitic acid esterified at position 2 is linearly related to the concentration in the original triglycerides; the slopes of the regression lines are roughly paralel but the fats show a widely divergent elevation. Linear but different 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 was found that the molar percentages of some fatty acids are closely interrelated within the positions of the triglycerides (Verbeke and De Brabander, 1979). Since we used in this study fats of diverse anatomical locations from animals on different feeding regimes the consistency of the results suggest that some of the observed relationships may be species specific.

Identification of animal fat species on basis of positional distribution patterns

On basis of the different relationships in the positional distribution of some fatty acids over the triglyceride species, appropriate parameters were calculated which gave a minimal variance within the species and a maximal difference between the species (Fisher, 1936). As shown in Fig. 2 pig fats are effectively discriminated from the other fats by the positional distribution of palmitic acid and the proportion of oleic acid within the triglycerides. Beef fat is differentiated from the horse + hen fat using stearic acid as parameter (Fig. 3). Hen fat is discriminated from horse fat in using parameters calculated on basis of the distribution of palmitic acid and palmitoleic acid over the triglycerides (Fig. 4). Through successive use of 4 discriminant parameters the fats can be classified in their correct groups.

Estimation of the relative composition of binary fat mixtures

Fat adulteration is evident if the parameters of an unknown fat fall in between the clusters of the fats studied. The confidence level of fat adulteration was studied using a computer program (see Experimental). Discriminant parameters of the different fats were represented in bivariate scatter diagrams and equal frequency ellipses containing either 95 %, 99 % or 99.9 % of the samples were calculated following Defrise-Gussenhoven's

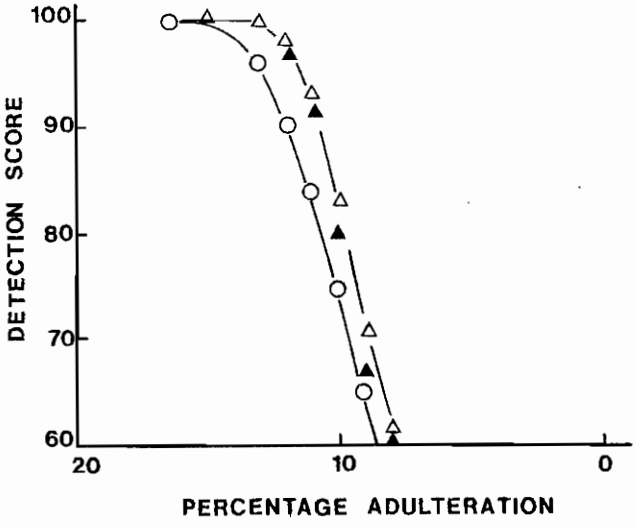

Fig. 5 Detection score in function of percentage adulteration of pig fat with beef (△), horse (▲) or hen (○) fat. (Discriminant parameters used : proportion palmitic acid and proportion oleic acid in the triglycerides).

TABLE 2 Discriminant parameters used in bivariate association for detection of the adulteration of animal fats.

Fat species	Adulterant	Discriminant parameters				r	Adulteration % detected (x)
			$x \pm s_x$		$y \pm s_y$		
Pork fat	Beef fat	PROP 16:0	83.26±5.71	PROP 18:1	10.08±1.93	0.693	11
	Horse fat	TG 14:0	1.95±0.37	3T-2M16:0	-51.98±3.65	-0.156	10
	Hen fat	TG 16:0	26.75±3.56	PROP18:1+2	10.65±2.46	-0.937	11
Beef fat	Pork fat	MG 18:0	11.99±3.46	PROP16:0+18:2	23.42±3.97	0.907	9
	Hen fat	TG 18:2	1.58±0.66	MG 18:2	2.8±1.19	0.893	15
Horse fat	Beef fat	TG 16:1	7.97±2.66	TG 18:0	3.38±0.9	-0.909	5
	Hen fat	MG 14:0	10.74±2.15	DG 18:2	3.3±1.34	0.442	30
Hen fat	Horse fat	MG 16:1	3.63±1.32	MG 18:3	0.46±0.47	-0.254	21

(x) : $p < 0.05$

procedure. Admixture of a determined percentage of another fat result in a new cluster which is shifted in respect to the first ellipse. The number of adulterated samples falling outside the equal frequency ellipse of the pure fat is calculated and termed detection score. In Fig. 5 the detection score at the 95 % confidence level of either beef, horse or hen fat in pig fat using a pair of discriminant parameters is represented in function of the percentage adulteration. From this graph it is evident that 90 % of the pig fat samples containing either 11 % beef, horse or hen fat will fall outside the 95 % equal frequency ellipse of pork fat and thus be classified as adulterated at the 5 % level of significance. The discriminant parameters for different combinations of fat adulteration, assuring a maximum of detection capability at the 95 % confidence level are summarized in Table 2. Use of these parameters allow estimation of low percentages (ca. 10 %) of pork fat in beef fat or horse fat in beef fat. However, admixture of low percentages of hen fat in horse fat or beef fat are more difficult to detect.

Differentiation of meat species on basis of fat species identification

Through fat analysis, the relative amounts of the meat species in the cooked meat product can be indirectly estimated. From the total fat content and pancreatic lipase analysis of the triglycerides isolated from the meat, the identity of the fats was determined. For fat mixtures the optimal discriminant parameters were selected and its position in the equal frequency ellipse calculated. The relative amount of fat species in the sample may be calculated from the relative location of the sample between the mean values of the two fat species. Assuming a typical fat percentage for one of the meat species, the relative composition of the meat species in the product is calculated. The detection limit of meat species in meat products depends critically upon the fat content of the meat used. In sausages, the high fat content of the added lard renders detection of other meat species difficult : up to 40 % lean meat of other species can be added before fat admixture is statistically detected.

CONCLUSION

Fatty acids are incorporated into the triglycerides according to a species specific pattern. It is shown that some of the relationships between the fatty acid distribution of the triglyceride molecule can be used as a reliable method in detecting fat adulteration. Since meat is always

associated with fat, this analysis can be used in identifying meat species in heated meat products.

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