4-63 APPLICATION OF A NEW METHOD FOR THE DETERMINATION OF FREE FATTY ACIDS IN MEAT LIPIDS

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The FFA_s are formed in complicated lipid systems either by hydrolytic breakdown of the molecules of glycerides,or by autooxidation of the side fatty acid chains. The hydrolytic and autooxidative breakdown of meat lipids is connected very often with developing off flavours upon the technological processing of meat, or with rancidity, in which case, secondary products of oxidation, dangerous for consumer health, are formed.

The official procedure for the detdrmination of free fatty acids content in meat and other food products, containing lipids,is based on the titration of the lipid extract with an alcoholic solution of potassium hydroxide. Using the Bligh and Dyer (1959) method,24 hours are necessary for the lipid extraction procedure.Beside that samples with very löw lipid content require large volumes to be extracted in order to obtain the necessary quantity of extracted fat. The total content of FFA_s is calculated as per cent oleic acid.

Out of the many procedures developed for the determination of FFA_s in vegetable

oils and fats, there are only few noted in literature which measure the individual FFA_s (Ottenstein, D.M., 1974, Chapman, G.W.Jr., 1979).

In this study we use Maxwell's method (1981) for the extraction of meat lipids by which the procedure takes no more than 30minutes. The isolation and identification of FFA_s in lipid extracts by the new method of Schwartz and Gadjeva (at press) is completed within 40 min. The sodium salts of FFA_s formed on the column are separated from the other classes of neutral lipids by rinsing with hexane. After passing HCl vapour through the column, liberated FFA_s are eluted with hexane and individually determined by GLC.

Applying the new method to the three groups of meat muscles we obtain quantitative results for FFA_s present in newtral lipid extracts of meat samples within very short period.

MATERIALS AND METHODS

Materials: M.longissimus dorsi muscles were removed from freshly obtained pig carcasses in the local slaughter house. Broilers were purchased from the local retailer. After skin removal and boning, breast and leg muscles were trimmed as much as possible from any visible fat and adipose tissue. Lean muscles were minced and put into plastic pouches separately and stored at $+4^{0}C$ until used for extraction, not later than within three hours.

Extraction of the total non polar lipids from meat muscles: The procedure of Maxwell (1981) with some modifications was used for lipid extraction from all muscles. 3-6g of meat weighed to the nearest 0,001g, were ground in a large mortar with 6g of anhydrous Na_2SO_4 and 6g of sand. Then, 12g Celite545 were added and reground for 2min. until a uniform mixture was obtained. The latter was transferred quantitatively into a glass column through a glass funnel. A stainless steel rod with a proper diameter was used to tamp the mixture into the column to obtain a uniform bed with a height of 50-60 mm. A volumetric flask (25ml) was set under the column for collecting the lipid extract. Hexane, distilled in glass was used as an extracting solvent. The lipid content of an aliquot was determined after evaporation of the solvent under nitrogen and heating. The lipid content of each sample was made uniform by delution or concentration of lipid extracts for convenience in the subsequent analysis of FFA_s content.

Procedure for the separation and identification of FFA_s in lipids from lean meat

An aliquot from the lipid extract in hexane, comtaining 67µg pentadecanoic asid as an internal standard, was passed through the small column (10mmi.d.) with Celite 545:sat.sol.Na_3PO_4 (2:1w/w) according to Schwartz, Gadjeva (at press). The FFA_s were arrested as their salts on the column, and the other constituents of lipids were eluted with hexane. The lipid eluent was checked for saponification by two solvents (benzene: hexane, 1:1), one dimensional TLC-method after spraying with a 20% sol. of $(NH_4)_2S\,O_4$ and heating.

The FFA_s on the column were recovered after passing vapours of HCl and prepared by the above-mentioned method for injection in a gas chromatograph. GLC analyses were performed on a "Varian 2100" gas chromatograph, equiped with a flame ionization detector. The glass column, 1,8m, 2mm i.d., was packed with 15%DEGS on Chromosorb 100-120mesh. The injector was set at $220^{0}C$; detector temperature-at $250^{0}C$, and column oven was maintained at $180^{0}C$. The carrier gas was nitrogen at a rate of 30ml/min.

RESULTS AND DISCUSSION

Tabled 1 shows the results obtained from the extraction procedure of neutral lipids from different fresh lean muscle groups. The ratio between total lipids in breast and leg muscles from broilers was 1:5. These data correspond well with those received by Pikul at al. (1984), despite of the fact that they used a mixed solvent system ($CHCl_3$:MeOH) for extraction.

Table1: Content of tital neutral lipids, isolated from fresh lean muscles

%	Type of muscles: Broilers Breast m.	Broilers Leg m.	Pork M.long. dorsi
Neutr.* lipids	0,345	1,67	4,23

* Data represent means of three different determinations

The present data obtained for the total neutral lipid extract from M.longissimus dorsi muscle are close to, but lower than those given by Marmer and Maxwell (1981), 4,97% in lean pork. Possibly, the reason for that could be in differences in the breeding conditions of animals and also in the types of muscle groups. In short, the good results obtained in the pre-

sent work by the modified column method of Maxwell,Marmer(1981) point out its advantages of a fast and reliable one compared to the time consuming procedure of Bligh,Dyer (1959). The relative concentration of FFA in meat lipids is low,Below 2%,Pikul(1984), but they are of a great importance for the stability and quality of meat during storage or the technological process because they can catalyse the processes of autooxidation.

Table 2 gives the percent composition of the individual FFA in meat lipids determined by applying the new procedure(Schwartz,Gadjeva,at press).For the first time FFA in meat lipids are identified quantitatively without applying previously separation of total lipids by TLC.Using Celite 545:Na_3PO_4 arrestant column the other classes of lipids passed through it unchanged with the hexane eluent under the conditions of the experiment. The absence of a spot coresponding to the FFA on TLC- plate where the lipid sol was applied after following out of the column, shows that the whole sum of them have been caught quantitatively on the column as their salts a saponification of the other classes has not occured. The composition and quantity of FFA in the neutral lipids of breast m. and leg m. from broilers are almost the same. The dominating saturated fatty acids in both are palmitic and stearic acids. Lauric and miristic acids are present in trace concentrations in both white and dark muscles of broilers. The contents of free polyunsaturated fatty acids are 15,59 and 14,33% in breast and leg muscle and the USFFA/SFFA ratio is 1,36 and 1,47 in the former and the latter,resp.These values are lower than those found by Pikul et al.(1984) in triacylglycerols of breast and leg muscles in chicken meat(24,11% in breast ,25,45% for dark meat),who have not investigated the lipid for the class of FFA.

Table 2

Percent composition of FFA in neutral lipids from fresh lean muscles

FFA	Type of muscles: Broilers Breast m. x ± sd		Broilers Leg m. x ± sd		Pork M.longiss. dorsi x ± sd	
12:0	t		t		1,03	0,15
14:0	t		t		3,00	0,15
16:0	31,60	0,20	30,26	0,15	28,56	0,66
16:1	7,60	0,20	10,23	0,15	4,83	0,10
18:0	10,36	0,15	10,30	0,10	11,99	0,09
18:1	34,40	0,10	34,96	0,15	37,59	0,09
18:2	14,36	0,15	13,70	0,20	10,80	0,10
18:3	1,23	0,05	0,63	0,11	0,77	0,03
USFFA	57,73		59,10		54,20	
USFFA / SFFA	1,36		1,47		1,18	
TFFA as wt% x10^{-3} per 100g wet tis.	20,3		108,6		29,4	

The lower percentage of USFFA found in the fraction of FFA isolated from M.long.d. and shows hydrolytic processes here go at different rates. The $\frac{FUSFA}{FSFA}$ ratio here is 1,18 which is lower than those found by Wood and Lister(1973): $\frac{FUSFA}{FSFA}$ = 1,53 in nonpolar lipids of the same muscle from pork meat. A possible explanation of this fact is that we have worked here with a M.l.d. muscle removed from pigs fed a special diet and have only investigated the fraction of FFA_s in neutral lipids.

As it is obvious from Table 2, the highest weight percentage of FFA_s in wet meat muscles is contained in neutral lipids isolated from leg muscle of broilers.Which confirms the findings of Bosung and Ganrot(1981) that lipolysis develops faster in dark-muscles and also that the higher percentage of triacylglycerols in broiler dark meat ensures a higher rate of hydrolysis.

The application of the new column- gas chromatographic method for determining FFA_s content in meat lipids has the following advantages:

(a) Quantitative determination of each fatty acid in lipids. By changes in each one we can follow indirectly the hydrolytic and oxidative processes occuring in meat which are of a great importance for its quality.

(b)The high sensitivity of the new method guarantees the detection and determination of FFA_s in amounts from 30 to 150ppm related to lipids.

(c) FFA_s content in lipids can be determined without the use of the widespread TLC methods which are laborious and time consuming.

(d) The fraction of FFA_s arrested on the Celite545:Na_3PO_4 column is free from other fractions which damage and shorten the life of GLC-column.

(e) When the rate of flowing out of the arrestant column is kept between 3-4 min.,the method guarantees the "clean" isolation of only FFA_s.No saponification occures

(f) The method is very suitable to be applied to meat and foods with low fat content.

LITERATURE

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