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Lipolysis and fractionation of triglycerides by Argentotion-TLC in lard and beef tallow mixtures.

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Abstract

Argentotion-TLC and enzymic lipolysis were applied for detection and evaluation of lard in some food products.

Triglycerides (TG) and B-monoglycerides (B-MG) of the pure lard and beef tallow as well as beef tallow mixtures with 1, 3, 5, 7, 9, 11, 13, 15, 17, and 19% of lard were analysed, including TLC separation based on chain length and polarity.

Feasibility of applying some calculation factors (palmitic acid envichment factor; unsaturation ratio; total C_{16}/total C_{18} fatty acids in B-MG; Saturated/unsaturated fatty acids in B-MG; and USU/SUS ratio) as a criteria for the detection of lard contamination were assessed.

The data showed that palmitic acid ($C_{16:0}$) identified in lard was mainly incorporated in B-monoglyceride, while oleic acid ($C_{18:1}$) and linoleic acid ($C_{18:2}$) were preferentially esterified in the 1- and 3- positions of the triglycerides. Palmitic acid envichment factors were 2.393 and 0.893 in lard and beef tallow, respectively. The unsaturation ratio in lard (0.389) was markedly lower than that in beef tallow (1.038).

Total C_{16}/total C_{18} fatty acids in B-MG ratios were 2.395 and 0.486; while, saturated/unsaturated fatty acids in B-MG. ratios were 2.930 and 0.486 in lard and beef tallow, respectively. Glyceride patterns showed that USU/SUS ratio was markedly higher in lard (27.301) than that in beef tallow (0.608). Fractionation of triglycerides by silver nitrate TLC showed that lard was characterized by 10 identified bands, whereas beef tallow recorded only 4 bands with higher R_F values.

Regarding to the afore-mentioned mixtures of lard and beef tallow, t could be noticed that palmitic acid envichment factor might be used to detect about 3% lard or more; unsaturation ratio 5% lard as more; C_{16}/C_{18} fatty acids ratio 13% lard or more; saturated/unsaturated fatty acids ratio 3% or more; and USU/SUS ratio 5% lard or more in beef tallow mixtures.

Introduction

The specific distribution of polmitic acid in the B-position of the triglycerides of lard offers a new analytical tool for evaluating lard-beef tallow mixtures.

Recently, Verbeke et al. (1979) showed that the different fatty acids incorporated in B-position of beef tallow and lard were closely correlated to the corresponding fatty acid contents in the total triglycerides. They mentioned that this relationship can be used to determine quantitatively the adulteration percentage of lard with beef tallow.

Materials and Methods

1- Materials:

Fat tissues:

All samples under study were taken from Oslo slaughter house immediately after slaughtering.

Lard was withdrawn from park outer back fat of male yorkshire animals. While, beef tallow was trimmed free from lean meat of male animals.

2- Analytical methods:

2.2. Fat extraction:

Fat was extracted from fatty tissues using the method described by Folch et al. (1957) as modified by Ways et al. (1964) using chloroform: methanol (2:1).

2.2. Preparation of triglycerides:

The triglycerides were separated from total fat by adopting the method of Dister and Baur (1965).

2.3. Preparation of B-monoglycerides:

Enzymatic preparation of B-monoglycerides from triglycerides by pancreatic lipase was performed as described by Rossell et al. (1978).

2.4. Preparation of methyl esters of fatty acids:

The methyl esters of fatty acids were prepared from triglycerides and B-monoglycerides using the method described by Rossell et al. (1983).

2.5. Gas Liquid Chromatography of methyl esters of fatty acids:

The methyl esters of fatty acids were separated using a PYE unicam (GCD) Gas Liquid chromatography apparatus with S 8 autosampler.

2.6. Factors calculation:

The polmitic acid envichment factor; the unsaturation ratio and other ratios based on the fatty acids composition of triglycerides and B-monoglycerides were calculated by the method used by Abdel-Fattah (1974). The following equations were used respectively:

(1) Palmitic acid enrichment factor = $\frac{\text{\% plamitic acid in B-MG.}}{\text{\% plamitic acid in T.G.}}$

(2) Unsaturation ratio = $\frac{\text{\% of unsaturated fatty acids in B-MG}}{\text{\% of unsaturated fatty acids in T.G.}}$

(3) a. $\frac{\text{\% of total } C_{16} \text{ fatty acids in B-MG.}}{\text{\% of total } C_{18} \text{ fatty acids in B-MG.}}$

b. $\frac{\text{\% of saturated fatty acids in B-MG}}{\text{\% of unsaturated fatty acids in B-MG}}$

2.7. Fractionation of triglycerides by silver nitrate-TLC:

Fractionation of triglycerides by silver nitrate-TLC was carried out as described by Gegiou et al. (1983).

Results and Discussion

Distribution of fatty acids within B-monoglycerides and triglycerides of lard and beef tallow:

Table (1) showed that the quantitative fatty acid composition in triglycerides markedly varied in lard than that in beef tallow. Besides, the data revealed that, in agreement with the previous findings reported by Hilditch et al. (1964); Mattson et al. (1964); Abd El-Fattah (1970); El-Dashlouty (1978) and Verbeke et al. (1979 b) palmitic acid ($C_{16:0}$) of lard is mainly incorporated in the B-position, while oleic acid ($C_{18:1}$) and linoleic acid ($C_{18:2}$) are preferentially esterified in the 1- and 3- positions of the triglycerides. Meanwhile, the unsaturated fatty acids tend to prefer Position-2, while palmitic acid ($C_{16:0}$) accumulates in positions 1 and 3 in beef tallow.

Table (2) revealed that the palmitic acid enrichment factor was 2.393, and 0.893 in lard and beef tallow, respectively. This may be due to the low content of palmitic acid in B-monoglyceride and its high content in triglyceride of beef tallow. On the contrary, the palmitic acid was rather high in the former than in the latter fractions of the lard. These results are in agreement with those reported by El-Dashlouty (1978); Abou-Arab (1980); and Nour El-Din et al. (1984).

While the unsaturation ratio was rather low in lard (0.389) than that in beef tallow (1.038). This may be due to high content of unsaturated fatty acids in B-monoglycerides and its low content in triglycerides in beef tallow, while lard recorded an opposite trend. These data are in close agreement with that previously mentioned by Amer et al. (1974); Abd el-Fattah (1974), El-Dashlouty (1978); Abou-Arab (1980) and Bayoumy (1982).

Table (1) : Fatty acid composilion of B-monoglycerides and triglycerides of lard and beef tallow.

% Fatty	Lard		Beef tallow	
acids	B-MG.*	T.G.**	B-MG.*	T.G.**
$C_{14:0}$	5.040	1.805	3.336	3.045
$C_{14:1}$	0.068	0.095	0.869	1.675
$C_{16:0}$	56.156	23.465	19.839	22.225
$C_{16:1}$	4.368	3.685	6.817	6.565
$C_{17:0}$	0.00	0.535	0.00	1.920
C_{18} Br.	0.00	0.455	0.00	1.085
$C_{18:0}$	6.968	11.500	5.469	12.240
$C_{18:1}$	12.978	39.685	44.772	43.065
$C_{18:2}$	5.326	11.720	4.663	2.835
$C_{18:3}+C_{20:1}$	0.526	3.630	1.785	2.135
$C_{22:1}$	0.00	1.040	0.00	0.500
unknown	0.00	0.670	0.00	0.365

* B-MG. = B-monoglycerides.

** T.G. = Triglycerides.

Table (2): Calculated factors of lard and beef tallow.

Calculated factors	Lard	Beef tallow
Plamitic and enrichment Factor	2.393	0.893
Unsaturation ratio	0.389	1.038
% Saturated Fatty acids/ unsaturated fatty acids	2.930	0.486
USU/SUS	27.301	0.608
C_{16}/C_{18}	2.395	0.486

The data revealed that the total C_{16}/total C_{18} ratio in B-monoglyceride of lard was considerably high (2.395); while it was comparatively low in beef tallow (0.486).

On the other hand, saturated/unsaturated fatty acids ratios in B-monoglycerides was 2.930; and 0.486 in lard and beef tallow, respectively. This may be due to high content of saturated fatty acids and low content of unsaturated fatty acids in B-monoglyceride of lard, while beef tallow recorded an opposite trend. Such data coincide with those previously reported by Abdel-Fattah, (197 a and 1974); El-Dashlouty, (1978) and Nour El-Din et al. (1984).

It is note-worthy that the usu/sus ratios was higher in lard than in beef tallow, as it was almost 45 fold that of the beef tallow (27.301 and 0.608) in lard and beef tallow, respectively.

This may be due to the fact that lard contains relatively high percentage of saturated fatty acids in B-monoglycerides (68.164%); while beef tallow contains rather low percentage of saturated fatty acids in B-monoglyceride (28.644%).

Such data are in good agreement with Amer et al., (1974); Bracco et al. (1976); El-Dashlouty (1978) and Nour El-Din et al. (1984) findings.

Generally, the usu/sus ratio may be recommended to be successfully used as a criteria for lard detection in beef tallow.

The data of the fatty acid composition of the triglycerides and B-monoglycerides of the certain mixture of lard and beef tallow revealed that there was a slight insignificant decrease in palmitic acid ($_{16:0}$) and stearic acid ($C_{18:0}$) in the triglycerides, while the same two fatty acids markedly increased as the lard percentage was increased in B-monoglycerides.

Besides, there was a slight insignificant decrease in $C_{14:1}$ and $C_{18:1}$ in the triglycerides, while there was a slight increase in the former acid and significant decrease in the latter acid in B-monoglycerides with the increament of lard percentage. Moreover, there was a slight increase in $C_{18:2}$ in both triglycerides and B-monoglycerides as the lard percentage was increased in the certain mixtures.

It is note-worthy that on the basis of these findings the detection of lard in other animal tallows may be performed which agrees with Abdel-Fattah (1970); Amer et al. (1974) and Bracco et al. (1976).

Furthermore, is clear that as the lard percentage increased a real increase of the palmitic acid enrichment factor was recorded. This may be due to the fact that 90% of the total plamitic acid in lard was in the B-position (Mattson et al. 1964; Amer et al. 1972; and Bracco et al. 1976).

In agreement with the results previously reported by Mattson et al. (1964); Abdel-Fattah (1970 and 1974); El-Dashlouty (1978) and Abou-Arab (1980) it is obvious that palmitic acid enrichment factor of lard is alwasy above 2.30, while it is lower than 0.80 in beef tallow. Furthermore, from obtained data it is evident that lard is easly detectable if it is present as contaminant at the rate of 3% or more in beef tallow.

Moreover, a decrease of the unsaturation ratio to 0.777 may indicate the presence of lard at 5% level or more in beef tallow mixtures.

Besides, the addition of lard to beef tallow resulted in a rather slight increase in the C_{16}/C_{18} fatty acids and saturated/unsaturated fatty acids ratios. This may be due to the relatively high and the markedly low corresponding ratios in both lard and beef tallow, 2.395 and 2.930, and 0.486 and 0.486; respectively.

An increase of the C_{16}/C_{18} fatty acids ratio above one may indicated the presence of lard at the level 13% or more. While, an increase of saturated/unsaturated fatty acids ratio to 0.652 or more may indicate the presence of lard at the level 3% or more.

These results are in good accordance with those reported by Abd el-Fattah, (1970 and 1974); El-Dashlouty, (1978); Abou-Arab, (1980) and Nour El-Din et al. (1984).

Moreover, the data revealed that the usu/sus ratio increased as the lard percentage was elevated. This may be attributed to the values of usu/sus ratio for lard.

Accordingly if the usu/sus ratio is 1.0 or more, this may indicate the presence of 5% lard or more. These results are in good agreement with those previously reported by El-Dashlouty, (1978), and Nour El-Din et al. (1984).

On the other hand, the fractionation of lard and beef tallow triglycerides were carried out by thin layer chromatography on plates of silica gel G impregnated with silver nitrate. Lard was characterized by 10 identified bands, whereas beef tallow illustrated only 4 bands with higher R_F values. These results are in agreement with those reported by El-Dashlouty (1978); Abou-Arab (1980) and Nour El-Din et al. (1984).

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