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APPLICATION OF GLC TECHNIQUE FOR CHARACTERIZATION OF LARD-BEEF TALLOW MIXTURES

(With 6 Tables)

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استخدام التحليل الكروماتوجرافى الغازى فى كشف دهن الخنزير فى خلطاته مع الدهن البقرى

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أجريت هذه الدراسه فى محاوله للتوصل الى أبسط الطرق التحليليه لتستعمل كمقياس للكشف عن دهن الخنزير مع الدهن البقرى.

تم دراسة تركيب الاحماض الدهنيه الموجوده فى الجلسريدات الثلاثيه وتلك الموجودة فى الموضع (بيتا) من الجلسريدات الأحادية الناتجه من التحليل الانزيمى لكل من دهن الخنزير والدهن البقرى والخلطات الناتجه من كليهما والتى تحتوى على ٣ ، ٦ ، ٩ ، ١٢ ، ١٥ ٪ دهن خنزير ، وذلك باستعمال التحليل الكروماتوجرافى الغازى. وقد أوضحت النتائج أن هناك نقص غير معنوى فى أحماض الميريستيك واللينولييك فى الجلسريدات الثلاثيه بزيادة دهن الخنزير . بينما حدث زيادة تدريجيه فى حامض البالمتيك فى الموضع (بيتا) من الجلسريداتالأحاديه بزيادة دهن الخنزير . وقد حدث نقص تدريجى فى أحماض الاستياريك والأولييك واللينولييك واللينولينيك فى الموضع (بيتا) من الجلسريدات الاحاديه وذلك بزيادة دهن الخنزير . كما حدث زيادة فى معامل التدعيم بحامض البالمتيك بزيادة نسبة دهن الخنزير ، بينما لوحظ نقص تدريجى فى نسبة عدم التشبع بزيادة نسبة دهن الخنزير . كما لوحظ زيادة فى نسبة الاحماض الدهنيه الكليه ك$_{١٦}$ / الاحماض الدهنيه الكليه ك$_{١٨}$ ، وكذلك نسبة الأحماض الدهنيه المشبعه / الاحماض الدهنيه غير المشبعه بزيادة نسبة دهن الخنزير .

فى ضوء ما سبق يوصى باستخدام المعادلات الحسابيه السابقه فى الكشف عن دهن الخنزير فى خلطاته مع الدهن البقرى.

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SUMMARY

Concerning the fatty acid composition of experimented standard mixtures of lard and beef tallow, the data revealed that there was a slight insignificant decrease in $C_{14:1}$, $C_{16:0}$ and $C_{18:2}$ in the triglycerides as the percent of lared increased, while there was a gradual significant increase in palmitic acid ($C_{16:0}$) in B-monoglycerides with the increment of lard percentage. On the other hand, there was a gradual decrease in stearic acid ($C_{18:0}$), oleic acid ($C_{18:1}$), linoleic acid ($C_{18:2}$) and linolenic acid ($C_{18:3}$) in B-monoglycerides as the lard percentage was increased. The palmitic acid enrichment factor was markedly increased as the percent of lard increased. It is always above 2.0 in lard, while it is lower than 0.85 in beef tallow. Furthermore, it is evident that lard is easily detectable if it is present at the rate of 3% or more in beef tallow, whereas the unsaturation ratio was gradually decreased as the percentage of lard was increased. This ratio was 1.4 or more in animal fats, while it reached 0.5 or less in lard. Moreover, a decrease of the unsaturation ratio to 1.01 may indicate the presence of lard at 9% or more in beef tallow mixtures. The total C_{16}/ total C_{18} fatty acids ratio revealed that the addition of lard to beef tallow resulted in a rather increase in this ratio. An increase to above 0.54 may indicate the presence of lard at the level 9% or more. The saturated/unsaturated fatty acids ratio showed gradually increase with the increment of lard percentage.

INTRODUCTION

Based on fatty acids distribution in individual triglycerides of natural fats, *Abdel-Fattah (1970 & 1974)* successfully detected lard in other animal fats through determination of the palmitic acid enrichment.

The fatty acid composition of beef tallow was determined by *Amer* et al. *(1974),* they found that beef tallow contains high percentages of saturated fatty acids. These fatty acids

were mainly located at the (1) and (3) positions while the (2) position was occupied by at least 65% unsaturated fatty acids.

El-Sayed and El-Dashlouty (1979) reported that the fatty acid composition of triglycerides and B-Monoglycerides of beef tallow was 0.7% and 2.4% Lauric, 4.1% and 12.2% Myristic, 4.6% and 1.9% Myristoleic, 33.9% and 23.9% Palmitic, 4.6% and 4.2% Palmitoleic, 19.9% and 7.0% Stearic, 24.4% and 33.8% Oleic, 7.8% and 14.6% Linoleic acid, respectively.

Somali and Sayed (1979) estimated lard in beef fat by Gas liquid chromatography, where the percentage of linoleic acid component of the major fatty acids along with the ratio of oleic acid to stearic acid has been used to detect it. They also estimated iodine value and refractive index in standard mixtures. They observed that 10% of lard in beef fat was not detectable by these values.

Verbeke et al (1979) showed that the incorporation of palmitic acid or oleic + linoleic acids into the 2- position of the triglycerides of lard and beef tallow to be highly correlated to corresponding acid contents of the triglycerides. On the basis of various regressions obtained for lard and beef tallow, the percentage of adulteration of lard with beef tallow may be determined.

For detection of a specific fats or fat substitutes in fatty foods, many trials have been made. However, there is no organized food proof scheme for the quantitative analysis of fats and problems of identifying individual fats and oils is quite complicated. This is particularly true in the case of mixture and processed fats *(Pomeranz & Meloan, 1987).*

MATERIAL AND METHODS

Material:

Fat tissue:

Beef tallow samples under study were taken from Assiut slaughter house immediately after slaughtering.

Lard was withdrown from pork outer back fat of male animals, while beef tallow was trimmed free from lean meat of male animals.

Methods:

Analytical methods:

1- Fat extraction:

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

2- Preparation of triglycerides:

The triglycetides were separated from total fat by adopting the method of *Dister and Baur (1965),* using column chromatography with silica gel (Merk 100-200 mesh) as adsorbent material and benzene eluting solvent.

3- preparation of B-monotriglycerides:

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

4- Preparation of methyl esters of fatty acids:

The methyl esters of fatty acids were prepared from aliquots of total lipids, triglycerides and B-monoglycerides using 5 ml 3% H_2So_4 in absolute methanol and 2ml benzene as mentioned by *Rossell* et al. *(1983).* The contents were sealed in special combustion tubes under nitrogen and heated for methanolysis at 90°C for 90 min. After cooling, phase separation was performed by addition of 5 ml water and methyl esters were extracted with 2 aliquots of 5 ml hexane each. The organic phase was discarded, filtered through anhydrous sodium sulfate to remove traces of water and concentrated by using rotary evaporator.

5- Gas liquid chromatograpay of methyl esters of fatty acids:

The methyl esters of fatty acids were separated using a PYE unicam (GCD) Gas liquid chromatography apparatus with 28 autosampler. The separation was performed with a column, 6 ft. Long and 2 mm O.D., packed with SP-233 on 100-120 mesh chromosorb WAW. The chromatographic analysis was carried out under the following condition: Column temp. program, 135°C and increased to 230°C by 16°C/min., and final hold for 8 min., injector temp. 240°C, detector temp. 260°C (FID) detector, carrier gas: Nitrogen 20 ml/min. The quantitative determination of the different acid was performed by measuring the peak areas with an Hewlett packard intergrator 3390A.

Factors calculation:

The palmitic acid enrichment factor, the unsaturation ratio and other ratios based on the fatty acids composition of triglycerides were calculated by the method used by *Abdel-Fattah (1974), El- Dashlouty (1978) and Bayoumy (1982).* The following equation were used respectively:

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

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

3- (a) $\frac{\%\text{ of total C16 fatty acid in B-MG}}{\%\text{ of total C18 fatty acid in B-MG}}$

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

RESULTS

The obtained results were recorded in Tables (1), (2), (3), (4), (5) and (6).

DISCUSSION

Fatty acid composition of experimental standard mixtures of lard and beef tallow:

The data of fatty acid composition of the triglycerides and B- monoglycerides of experimental standard mixtures of lard and beef tallow are shown in Tables (1 and 2). The data revealed that there was a slight insignificant decrease in $C_{14:1}$, $C_{16:0}$ and $C_{18:2}$ in the triglycerides, while there was significant increase in $C_{18:1}$ in the triglycerides as the percent of lard increased. On the other hand, it could be noticed that there was a gradual significant increase in palmitic acid $C_{16:0}$ in B- monoglycerides with the increment of lard percentage. Moreover, there was a gradual decrease in stearic acid ($C_{18:0}$), oleic acide ($C_{18:1}$), Linoleic acid ($C_{18:2}$)and Linolenic acid ($C_{18:3}$) in B- monoglycerides as the lard percentage was increased in the experimental standard mixture. It is noteworthy that on the basis of such data, the detection of lard in other animal tallow might be performed which coincides with *Amer et al. (1974); Bracco and Winter (1976)and Rashwan (1986).*

Palmitic acid enrichment factor:

An accurate parameter for detection of lard in animal fats was achieved by calculating the palmitic acid enrichment factor. Table (3) indicates the palmitic acid enrichment factor for experimental standard mixtures of lard and beef tallow. This factor was markedly increased as the percent of lard increased. This may be due to the fact that 90% of the total palmitic acid in lard was in B-monoglycerides (*Mattson et al., 1964; Amer et al., 1972 and Bracco and Winter, 1976).* In agreement with the data previously reported by several authors, it should be pointed out that palmitic acid enrichment factor of lard is always above 2.0, while it is lower than o.85 in

beef tallow. Furthermore, from Table (3) it is evident that lard is easily detectable if it is present at the rate of 3% or more in beef tallow.

The unsaturation ratio:

Results given in Table (4) showed that the unsaturation ratio of the experimental standard mixtures of lard and beef tallow gradually decreased as the percentage of lard was increased. The unsaturation ratio was suggested by *Amer* et al.*(1974); Abdel-Fattah (1974) and Rashwan (1986)* as a helpful method for detection of lard in animal fats. This ratio was 1.4 or mor in animal fats, while it reached 0.5 or less in lard. Moreover, a decrease of the unsaturation ratio to 1.01 may indicate the presence of lard at 9% or more in beef tallow mixtures.

Total C_{16} / total C_{18} Fatty acids ratio:

Table (5) shows the total C_{16}/ total C_{18} fatty acid ratio in experimental standard mixtures of lard and beef tallow. Such data revealed that the addition of lard to beef tallow resulted in a rather increase in the C_{16} / C_{18} fatty acids ratio. This might be due to the relatively high and markedly low corresponding ratio in both lard and beef tallow, 1.85 and 0.34, respectively. An increase of this ratio above 0.54 (Table 5) may indicate the presence of lard at the level 9% or more. Generally, this ratio could be successfully used as a helpful guide for detecting lard in pure beef tallow, as it was elevated as lard percentage was increased. These results are in good accordance with those reported by *El-Dashlouty (1978); Abou-Arab (1980); Nour el-Din* et al. *(1984) and Rashwan (1986).*

The saturated / unsaturated fatty acids ratio:

The saturated / unsaturated fatty acids ratios of experimental standard mixtures of lard and beef tallow are shown in Table (6). The results showed that this ratio was gradually increased with the increment of lard percentage. These results are in quite agreement with those previously reported by *Youssef and Rashwan (1989) and El-Zeini (1991).*

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Table (1): The percentage of fatty acids content in triglycerides of experimental standard mixtures of lard and beef tallow.

Fat mixtures (W/W)		% of fatty acid content							
Lard %	Beef Tallow	C14:0	C14:1	C16:0	C16:1	C18:0	C18:1	C18:2	C18:3
3	97	1.60	1.21	23.80	1.29	12.13	47.21	6.31	6.45
6	94	1.52	0.19	24.02	1.42	12.66	48.42	6.21	6.32
9	91	1.41	0.15	23.16	2.04	12.80	48.61	5.81	6.02
12	88	1.04	0.12	22.63	1.91	12.02	49.62	5.74	6.92
15	85	0.90	0.73	22.58	1.72	9.42	49.71	6.31	8.75

Table (2): The percentage of fatty acids content in B-monoglycerides of experimental standard mixtures of lard and beef tallow.

Fat mixtures (W/W)		% of fatty acid content							
Lard %	Beef Tallow	C14:0	C14:1	C16:0	C16:1	C18:0	C18:1	C18:2	C18:3
3	97	4.43	7.30	21.02	3.69	8.23	43.52	6.34	5.27
6	94	4.72	12.35	21.86	4.11	7.54	41.02	4.01	4.39
9	91	4.96	11.77	24.78	4.31	6.98	37.92	4.71	4.21
12	88	3.03	9.99	32.65	3.02	5.92	37.97	4.20	2.91
15	85	5.40	15.68	32.97	2.86	3.81	33.57	3.75	2.10

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Table (3): Palmitic acid enrichment factor for experimental standard mixtures of lard and beef tallow.

Fat mixture	Palmitic acid in B-MG	Palmitic acid in TG	Factor
3%	21.02	23.80	0.88
6%	21.86	24.02	0.91
9%	24.78	23.16	1.07
12%	32.65	22.63	1.44
15%	32.97	22.58	1.46

Table (4): The unsaturation ratio of experimental standard mixtures of lard and beef tallow.

Fat mixtures	U.S.F.A. in B-MG*	U.S.F.A in TG**	Ratio
3%	66.32	62.47	1.06
6%	65.88	62.56	1.05
9%	62.92	62.63	1.01
12%	58.09	64.31	0.90
15%	57.96	67.22	0.86

* U.S.F.A. = Saturated fatty acids in Beta-monoglyceride.

** U.S.F.A. = Unsaturated fatty acids in triglyceride.

Table (5): The total C_{16} / total C_{18} fatty acids ratio of experimental standard mixtures of lard and beef tallow.

Fat mixtures	Total C_{16} in B-MG	Total C_{18} in B-MG	Ratio
3%	24.71	63.36	0.39
6%	25.97	56.96	0.46
9%	29.09	53.82	0.54
12%	35.67	51.00	0.70
15%	35.83	43.23	0.83

Table (6): The S.F.A./ U.S.F.A. ratio of experimental standard mixtures of lard and beef tallow.

Fat mixtures	% S.F.A. in B-MG	% U.S.F.A. in B-MG	Ratio
3%	33.68	66.32	0.51
6%	34.12	65.88	0.52
9%	36.72	62.92	0.58
12%	41.60	58.09	0.72
15%	42.18	57.96	0.73