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DETECTION OF LARD ADULTERATION IN PURE BEEF TALLOW

(With 4 Tables & One Fig)

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الكشف عن غش الدهن البقرى بدهن الخنزير

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اجريت هذه الدراسه فى محاوله للتوصل بطريقة سهله يمكن استخدامها فى معامل مراقبة الجودة على الأغذية للكشف عن غش الدهن البقرى بدهن الخنزير .

استخدم فى هذا البحث طريقة الكروماتوجرافى الغازى لدراسة الأحماض الدهنيه فى الدهن البقرى ، ودهن الخنزير وكذا فى كل من الجلسريدات الثلاثيه والجلسريدات الأحادية الناتجه من كليهما. وقد أوضحت النتائج التى حصل عليها أن نسبة الاحماض الدهنيه غير المشبعه فى دهن الخنزير أعلى من الدهن البقرى، بالاضافه الى أن نسبة حامض اللينولييك (ك ١٨ : ٢) فى دهن الخنزير أعلى من مثيله فى الدهن البقرى. وبأستخدام بعض المعادلات الحسابيه المستنبطه من الأحماض الدهنيه اتضح أن معامل التدعيم بحامض البالمتيك يمثل ٨٥ر . فى الدهن البقرى بينما يمثل ٢ر٠٦ فى دهن الخنزير ونسبة عدم التشبع منخفضه فى دهن الخنزير (٥١ر٠) عنها فى الدهن البقرى (١ر٠٩) وأن نسبة الأحماض الدهنيه الكليه (ك / ك) فى ٢- أحادى الجلسرين مرتفعه فى دهن الخنزير (١ر٨٥) عنها فى الدهن البقرى (٣٤ر٠)وكذلك نسبة الأحماض المشبعه / الأحماض الدهنيه غير المشبعه كانت مرتفعه فى دهن الخنزير (٧٧ر٠) عنها فى الدهن البقرى (٥١ر٠). وبالاضافه الى ماسبق أمكن بأستخدام منحنى قياسى يوضح العلاقه بين نسب حامض اللينولييك ونسبة دهن الخنزير معرفة تركيز أو نسبة دهن الخنزير فى العينات المختلفه بمعلومية نسبة حامض اللينولييك.

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SUMMARY

This investigation was carried out in an attempt to find a reliable method which can be used in a quality control laboratory for the detection of lard adulteration in beef tallow. The laboratory technique is based on the Gas Liquid Chromatographic analysis of fatty acids composition in total extracted fat as well as in triglycerides and B-monoglycerides. The adulteration of the studied beef tallow samples with lard is made from some calculated ratios depending on the fatty acids composition of pure lard and beef tallow. The obtained results showed that lard contained more unsaturated fatty acids (66.53%) than beef tallow (45.38%), while total saturated fatty acids was much higher in beef tallow (52.59%) than in lard (31.97%). In addition, the stearic acid ($C_{18:0}$) was lower in lard (12.33%) than in beef tallow (16.40%) and the linoleic acid ($C_{18:2}$) in lard and beef tallow was found to be 14.79% and 3.39%, respectively. The saturated fatty acids/unsaturated fatty acids ratio was much higher in beef tallow (1.1) than in lard (0.48). Palmitic acid ($C_{16:0}$) of lard is mainly incorporated in B-monoglycerides (85.37%), while oleic acid ($C_{18:1}$) was the predominated acid in B-monoglycerides of beef tallow (48.33%). On the other side, oleic acid ($C_{18:1}$) was highly concentrated in the triglycerides of either beef tallow and lard (46.23% and 47.41%), respectively. Palmitic acid enrichment factor was 0.85 and 2.06 in beef tallow and lard, respectively. The unsaturation ratio was rather low in lard 0.51) than in beef tallow (1.09), while the total C_{16}/total C_{18} fatty acids in B-monoglycerides was much higher in lard (1.85) than in beef tallow (0.34), also the percent of saturated/unsaturated fatty acids in B-monoglycerides was rather high in lard (0.77) as compared with that in beef (0.51). Estimation of lard in unknown samples, is made by determining the percentage of linoleic acid content and taking the corresponding percentage of lard from the standard curve.

INTRODUCTION

Adulteration of fats represents a major problem to food law enforcement agencies and quality control Laboratory. Adulteration of fat is illegally practiced by using less expensive types of fat and oiles.

Moreover, in Islam and some other religions, adulteration of meat products by pork or lard is prohibited. Lard is responsible for many metabolic disorders in human such as arteriosclerosis and formation of gall bladder stones. For these facts meat and meat products must be subjected to some investigational analysis to prove to what extent they are free from or contain adulterants.

Nowadays, great attention is paid to find out more definite and modern chemical methods for detection of lard in fat and oil products. Therefore, various specific chemical methods are assessed.

Depenging on the fact that lard is the only fat which contains high percentage (more than 80% of saturated fatty acids at B-monoglycerides, the detection of lard in animal fats was achieved by calculating a proposed value called the unsaturation ratio, iodine value of B-monoglyceride iodine value of *Triglyceride (Amer et al., 1974).*

This ratio was found to be 1.4 or more in animal and vegetable oils and fats, whereas being 0.5 or less in lard. Values below 1.4 indicate the presence of lard in animal fats *(Amer et al., 1972 & 1974).*

Recently, *Verbeke et al. (1979)* showed that the different fatty acids incorporated in position 2 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. On the other hand, adulteration can be detected by the changed ratios of some fatty acids in lipid extracts *(farag et al., 1980).*

Khalil (1987) found that the major fatty acid in lard was oleic acid (39.03%) followed by linoleic acid (7.93%). After pancreatic lipase hydrolysis the author found that the major fatty acid in the B-Position was palmitic acid (62.64%) followed by oleic acid (16.79%), stearic acid (8.99%) and myristic acid (4.86%).

MATERIAL AND METHODS

Material:

Fat Tissue:

Beef tallow samples under study were taken from Assiut Slaughter hous 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 triglycerides were separated from total fat by adopting the method of *Dister and Baur (1965),* using column chromatography with silica gel (mark 100-200 mesh) as adsorbent material and benzene eluting solvent.

3- Preparation of B-monotriglycerides:

Enzymatic preparation of B-monoglycerides from triglycerides by pancreatic lipase 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 2 ml benzene as mentioned by *Rossellet* 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 throgh anhydrous sodium sulfate to remove traces of water and concentrated by using rotary evaporator.

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 58 autosampler. The separation was performed with a glass column, 6 ft. long and 2 mm O.D.,packed with SP-2330 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. 260°C (FID) detector, carrier gas: nitrogen 20 ml/min. The quantitative determination of the different acid

was performed the peak areas with an Hewlett Packard integrator 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 equations were used rspectively:

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) $\frac{\%\text{of total } C_{16} \text{ fatty acid in B-MG}}{\%\text{of total } C_{18}\text{fatty acid in B-MG}}$

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

Results

The obtained results were recorded in Tables (1), (2), (3) & (4).

Discussion

Fatty acid composition of pure lard and beef tallow:

The GLC analysis of fatty acids composition of pure lard and beef tallow are given in Table (1).

The data showed in Table (2) pointed that lard contained more unsaturated fatty acids (66.53%) than beef tallow (47.38%). On the contrary, total saturated fatty acids was much higher in beef tallow (52.59%) than that in lard (31.97%). Besides, the stearic acid ($C_{18:0}$) was lower in lard (12.33%) than in beef tallow (16,45%), while, the linoleic acid ($C_{18:2}$) in lard and beef tallow was found to be 13.79% and 3.39%, respectively.

An alternative check of the adulteration of the studied beef tallow samples with lard is made from calculated ratios depending on the fatty acids composition of pure lard and beef tallow. Table (2) showed that the S.F.A./U.F.A. ratio was much higher in beef tallow (1.11) than that in lard (0.48). This results might be due to that lard contained more unsaturated fatty acids than beef tallow. These findings are in good agreement with those reported by *El-Dashlouty (1978); Najari et al.* (1986); Rashwan (1986) and EL-Zeini (1991).

Another index of adulteration was derived from the $C_{18:0}/C_{18:2}$ ratio. This ratio amounted to 0.89 and 4.84 in lard and beef tallow, respectively. This results could be due to that the stearic acid ($C_{18:0}$) content was markedly lower in lard than that in beef tallow. On the other hand, the linoleic acid ($C_{18:2}$) content was much higher in lard than that in beef tallow. These results are in close agreement with that previously reported by *Rashwan (1986) and Youssef and Rashwan (1989).*

A calibration graph as illustrated in fig. (1) was plotted using the percentage of lard in beef tallow against the percentage of linoleic acid content in the experimental standard mixtures of lard in beef tallow. Estimation of lard in unknown sampes, is made by determining the percentage of linoleic acid content and taking the corresponding percentage of lard from the graph.

It is possible to determine the extent of lard to beef tallow by applying a simple regression equation: $Y = A + BX$

where: Y = percentage of lard.
A = Constant value.
B = Regression coefficient.
X = Concentration of linoleic acid.

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

The fatty acids composition of B-monoglycerides and triglycerides of pure lard and beef tallow are shown in Table (3)

Generally, Table (3) revealed that the quantitative fatty acids composition markedly varied in lard than that in beef tallow.

Palmitic acid ($C_{16:0}$) of lard is mainly incorporated in the B-monoglycerides (58.37%). Meanwhile, the same acid ($C_{16:0}$) behaves an opposite trend in beef tallow. Oleic ($C_{18:1}$) is the predominate acid in B-monoglycerides of beef tallow (48.33%).

On the other side, as shown in Table (3) oleic acid ($C_{18:1}$) was highly concentrated in the triglycerides of either beef tallow or lard (46.23% and 47.41%, respectively). Therefore, the distrebution of fatty acids in B-monoglycerides might be helpful method for the species of animal fats rather than that of triglycerides. These data coincide with the previous findings reported by *Abdel- Fattah (1970); Verbeke et al. (1979); Rashwan (1986) and EL-Zeini (1991).* They stated that palmitic acid ($C_{16:0}$) is mainly incorporated in B-monoglycerides, while oleic acid ($C_{18:1}$) predominates in the 1- and 3- positions of triglycerides.

Palmitic acid enrichment factor:

Table (4) showed that palmitic acid enrichment factor was 0.85 and 2.06 in beef tallow and lard, respectively. This may by due to low content of palmitic acid in B-monoglycerides and its high content in the triglycerides of beef tallow. On the contrary, The palmitic acid was rather high in the B-monoglycerides than in the triglycerides of lard. These results are nearly similar to those reported by *Abou-Arab (1980); Nour El-Din et al. (1984) and Youssef and Rashwan (1989).*

The unsaturation ratio:

Table (4) represents the data of unsaturation ratio of lard and beef tallow. Such data revealed that the suggested ratio was rather low in lard (0.51) than that in beef tallow (1.09). This might be due to high amount of unsaturated fatty acids in B-monoglycerides and it's low content in triglycerides of beef tallow, while lard recorded an opposite configuration. These data are coincided with those suggested by *Abdel-Fattah (1974); Amer et al.* (1974); Bayoumy (1982) and Rashwan (1986).

Total C_{16}/ total C_{18} fatty acids in B-monoglycerides:

The total C_{16}/ total C_{18} fatty acids in B-monoglycerides of lard and beef tallow are tabulated in Table (4). The data revealed that this ratio was much higher in lard (1.85) than that in beef tallow (0.34). This may be due to the fact that palmitic acid ($C_{16:0}$) is mainly incorporated in B-monoglycerides of lard. Similar observations have been reported by *El-Dashlouty (1978); Nour El-Din et al. (1984); Rashwan (1986) and El-Zeini (1991).*

Saturated / unsaturated fatty acids in B-monoglycerides:

Table (4) showed that the percent of S.F.A./ U.F.A. in B-monoglycerides was rather high in lard (0.77) as compared with that in beef tallow (0.51). This may be due the high content of saturated fatty acids and low content of unsaturated fatty acids in B-monoglycerides of lard, while beef tallow recorded an opposite tread. Such data coincide with those previously reported by *Abdel-Fattah (1974); Nour El-Din et al.(1984); Rashwan (1986); Youssef and Rashwan (1989) and El-Zeini (1991).*

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Fig. (1): Calibration of standard mixture of lard in beef tallow.

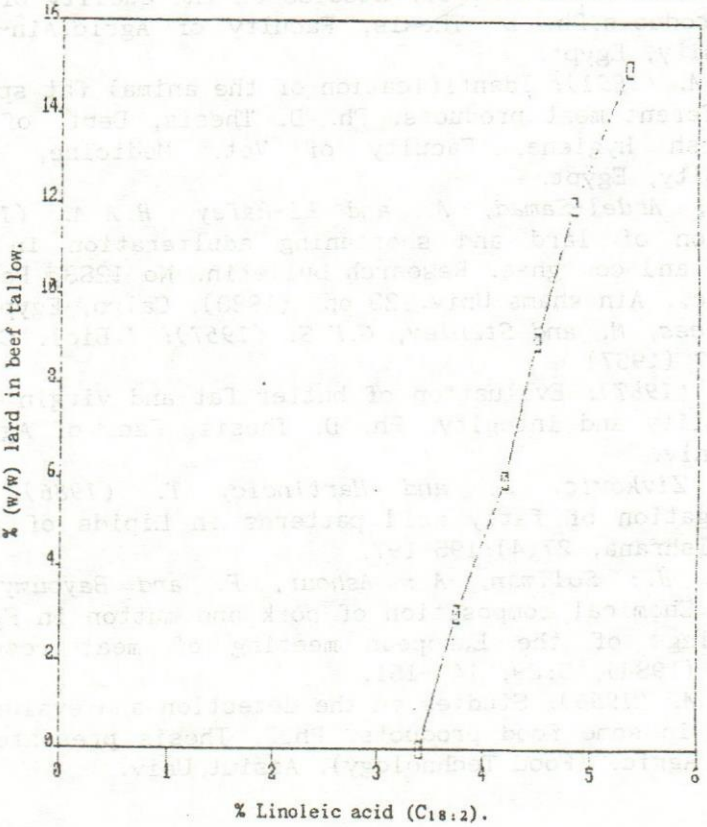

Table (1): GLC analysis of fatty acids composition of pure lard and beef tallow (% of the total).

Fatty acids	C14:0	C16:0	C16:1	C18:0	C18:1	C18:2	C18:3	C22:1
Beef tallow	6.47	29.72	8.39	16.40	30.60	3.39	2.02	2.98
Lard	0.16	19.48	8.76	12.33	37.01	13.79	4.38	2.59

Table (2): Some calculated ratios from fatty acids composition of pure lard and beef tallow.

Ratio	T.S.F.A.*	T.U.F.A.**	S.F.A. / U.F.A.	C18:0 / C18:2
Beef tallow	52.59	47.38	1.11	4.84
Lard	31.97	66.53	0.48	0.89

* T.S.F.A. = Total saturated fatty acids.

** T.U.F.A. = Total unsaturated fatty acids.

Table (3): GLC analysis of fatty acids composition of B-monoglycerides and triglycerides of pure lard and beef tallow.

Fatty acids	Carbon chain	Beef tallow		Lard	
		B-MG*	TG**	B-MG*	TG**
Myristic acid	C14:0	4.79	1.82	6.06	1.31
Myristoleic acid	C14:1	0.66	0.14	0.81	0.09
Palmitic acid	C16:0	20.12	23.61	58.37	28.32
Palmitoleic acid	C16:1	3.71	1.22	2.15	2.62
Stearic acid	C18:0	8.42	12.01	6.88	13.77
Oleic acid	C18:1	48.33	46.23	19.02	47.41
Lenoleic acid	C18:2	6.91	5.02	4.13	4.56
Lenolenic acid	C18:3	5.81	7.44	2.68	1.98

* B-MG = B-monoglycerides.

** TG = triglycerides.

Table (4): Some calculated ratios from fatty acids of B-monoglycerides and triglycerides of pure lard and beef tallow.

Ratios	Palmitic acid enrichment factor	Unsaturation	% total C16 in B-MG / % total C18 in B-MG	% S.F.A. in B-MG / % U.F.A. in B-MG
Beef tallow	0.85	1.09	0.34	0.51
Lard	2.06	0.51	1.85	0.77