World Academy of Science, Engineering and Technology
International Journal of Nutrition and Food Engineering
Vol:8, No:11, 2014

# Detection of Lard in Binary Animal Fats and Vegetable Oils Mixtures and in Some Commercial Processed Foods

H. A. Al-Kahtani, A. A. Abou Arab, M. Asif

Open Science Index, Nutrition and Food Engineering Vol:8, No:11, 2014 publications.waset.org/10000133/pdf

***Abstract*—**Animal fats (camel, sheep, goat, rabbit and chicken) and vegetable oils (corn, sunflower, palm oil and olive oil) were substituted with different proportions (1, 5, 10 and 20%) of lard. Fatty acid composition in TG and 2-MG were determined using lipase hydrolysis and gas chromatography before and after adulteration. Results indicated that, genuine lard had a high proportion (60.97%) of the total palmitic acid at 2-MG. However, it was 8.70%, 16.40%, 11.38%, 10.57%, 29.97 and 8.97% for camel, beef, sheep, goat, rabbit and chicken, respectively. It could be noticed also the position-2-MG is mostly occupied by unsaturated fatty acids among all tested fats except lard. Vegetable oils (corn, sunflower, palm oil and olive oil) revealed that the levels of palmitic acid esterifies at 2-MG position was 6.84, 1.43, 9.86 and 1.70%, respectively. It could be observed also the studied oils had a higher level of unsaturated fatty acids in the same position, compared with animal fats under investigation. Moreover, palmitic acid esterifies at 2-MG and PAEF increased gradually as the substituted levels increased among all tested fat and oil samples. Statistical analysis showed that the PAEF correlated well with lard level. The detection of lard in some commercial processed foods (5 French fries, 4 Butter fats, 5 processed meat and 6 candy samples) was carried out. Results revealed that 2 samples of French fries and 4 samples of processed meat contained lard due to their higher PAEF, while butter fat and candy were free of lard.

***Keywords*—**Lard, adulteration, PAEF, goat, triglycerides.

## I. Introduction

LARD, one type of animal fats, is frequently used with other plant oils such as olive and palm oils to produce shortenings, margarines and other specialty food oils [1]. Lard can be taken into account from 2 points of view, economical and religion. In certain countries, the producers of food products prefer to blend vegetable oils with lard in order to reduce the production cost [2]. Any food products containing lard is not allowed due to religious reason. Some religions like Islam, Judaism and Hinduism not allowed their followers to consume any foods containing lard in its formulation [3]; consequently, analytical method for detecting lard is highly needed.

Al-Kahtani H.A is with Food Science and Nutrition Dept., Faculty of Food and Agriculture Sciences, King Saud University, P.O. Box 2460, Riyadh 11451 (e-mail: hkahtani@ksu.edu.sa).

Abou Arab, A.A is with Dept. of Food Science and Technology, Faculty of Agriculture, Ain Shams University, P.O. Box 1241 , 86 HadaK Shoubra, Cairo, Egypt (Corresponding author e-mail: atefanwar@hotmail.com).

Asif M is with Food Science and Nutrition Dept., Faculty of Food and Agriculture Sciences, King Saud University, P.O. Box 2460, Riyadh 11451.

Identification of fats in general is achieved using a variety of features, the most important of which is the fatty acid composition. The fatty acid composition of animal fats, unlike oils varies widely within the same fat depending on many factors including the nutritional status and feeding habits of animals [4], animal breeds and the anatomical locality within the animal from which the fat was taken [5], [6].

The detection of lard may become more difficult when it is present as a minor component in other oils and fats. Therefore, methods dealing with the overall fatty acid composition may sometimes not be useful for detecting of lard. This necessitated the need to look into the FA distribution pattern within the triacylglycerol (TAG) molecules. Hence, determination of positional distribution of fatty acids was considered as an alternative option for this purpose. Previous investigations revealed that in spite of natural variations in fatty acid composition within the same fat yet distribution of some fatty acids in the triacylglycerol molecules remains almost unchanged [4].

The distribution of fatty acids within the triacylglycerols obtained by lipase hydrolysis, TLC and GLC [7], [8], [6] was carried out. Based on these data of distribution, palmitic acid enrichment factor (PAEF) and unsaturation ratio were recommended by [9] for the detection of lard in other animal fats. However, Youssef et al. [10] used these factors to detect lard in beef fat blends, canned luncheon meat and sausage samples imported from different countries.

In previous reports, application of lipases and gas liquid chromatography in structural studies of natural TAG molecules has been discussed [1], [11], [12]. Pancreatic lipase hydrolysis is a useful technique in positional analysis of FA distribution, particularly at the sn-2 position of TAG molecules. Application of this technique has shown that in most fats and oils, the middle (sn-2) position is preferentially occupied by unsaturated fatty acids and the only exception is lard, in which the sn-2 position is predominantly occupied by saturated fatty acids, particularly palmitic acid [13]. Therefore, this unique feature of lard was used to determine lard adulteration in food systems [14]. Saeed et al. [15] used this technique to detect adulteration of beef, mutton and chicken products with pork. Similarly, Soliman and Younes [16] have demonstrated the usefulness of the technique for determining adulteration of butterfat with either beef tallow (BT) or cottonseed oil.

In this study, the pancreatic lipolysis technique and gas liquid chromatography were adopted to monitor the

Open Science Index, Nutrition and Food Engineering Vol:8, No:11, 2014 publications.waset.org/10000133/pdf

compositional variations in sn-2 positional fatty acids in some vegetable oils (corn, sunflower, olive and palm oil) and animal fats such as camel, beef, sheep, goat, rabbit and chicken after adulteration with different levels of lard (1, 5, 10 and 20%). This study attempted to use a multivariate data analysis approach to evaluate results and find a way to discriminate lard adulterations from other animal fat (AF) adulterations.

## II. Materials and Methods

### A. Materials

Animal fats, which are likely adulterated with lard, namely, camel, sheep, beef, goat, rabbit, and chicken, were selected and obtained from local slaughter houses in Riyadh City, Saudi Arabia. Rabbit fat was obtained from El-Taybat farm in AL-Kharj area, KSA. Lard was purchased from a hypermarket in Kawal Lampor, Malaysia. Vegetable oils such as corn, sunflower, palm oil and olive oil were purchased from Riyadh city, Saudi Arabia.

### B. Preparation of Animal Fats

The collected samples of animal fats were prepared from the under skin fat and adipose tissues of freshly slaughtered animals. The fats were scraped for the removal of non-fatty tissues, and then washed well with water to remove any hair. The cleaned samples were cut into small pieces before extraction (wet rendering) and the separated fat was dried over sodium sulfate anhydrous. The dried fat samples were then kept in refrigerator (~5°C) until use.

### C. Preparation of Fat and Oil Mixtures

Fat (camel, beef, sheep, goat, rabbit and Chicken) and oils (corn, sunflower, palm and olive) were substituted with 1, 5, 10 and 20% lard. The mixtures of fats and oils mixed well, then stored in refrigerator until uses.

### D. Preparation of Triacylglcerols (TAGs)

Preparation of TAGs from extracted fat was undertaken according to [17] using column chromatography as follow: The column, 290 mm in length with 19mm internal diameter was properly packed with a slurry of 30 gm silica gel 70-230 mesh (Merck) activated at 120°C to constant weight in benzene. After settle down of the silica gel, about 1.0 gm of dried fat or oil was dissolved in 2 ml chloroform, then introduced carefully into the top of the column and the sample was allowed to adsorb on the surface of silica gel for about 3 min. The column was eluted with 200ml benzene, the elute was collected, and the solvent was evaporated under vacuum using rotary evaporator (Büchi EL131, Switzerland).

### E. Preparation of Fatty Acid Methyl Esters

Fatty acid methyl esters were prepared according to [18] using BF3/methanol reagent (14% Boron Trifluoride), and were analyzed by gas chromatography (Shimadzu, 2010) equipped with a flame-ionization detector (FID). Gas chromatography operation parameters were as follows: Column (FAMEWAX, 30M, 0.32µm ID, 0.25µm Inlet: 250°C), Detector (FID, 300°C), Column Flow 1.5Ml/min. Velocity: 33.232 CM/SEC. Oven Prog. 190°C (Hold 4 min) to 225°C /4°C/min. Peaks were identified by comparison with fatty acid methyl ester standards (Sigma Chemicals, St. Louis, MO, USA).

### F. Pancreatic Lipolysis of Triglycerides

Pancreatic lipolysis of TG was carried out according to [19] as follow: 100 mg of triglyceride was emulsified with 20 ml of aqueous gum acacia solution (10gm gum acacia, 8 gm ammonium chloride, 2.2 gm calcium chloride, 0.5ml 0.05% bile salt, then dissolved in 100ml distilled water). The pH of the emulsion was adjusted to 8.2 using ammonium hydroxide 4N. The temperature of the emulsion was adjusted to 40°C. Whilst stirring, 0.2-0.3 gm of lipase (lipase from porcine, Sigma) was added and stirring for 30 min. The reaction was stopped and extraction of hydrolysate was achieved three times with 20 ml diethyl ether, and then evaporates under nitrogen. The lipolysate was dissolved in 2ml chloroform.

### G.TLC-Fractionation of 2-MG

The content of 2-MG in the hydrolysate could be separated on boric acid (0.4N) impregnated thin layers of silica gel. Plates were activated at 110°C for 2 h. The hydrolysate solution in chloroform was applied as a band, and developed with a solvent mixtures chloroform-acetone (96:4v/v). The developed plates were air dried and spraying with 0.1% of 2, 7-dichlorofluorescin in 95% in ethanol, then visualized under ultraviolet light. TLC references standard was used to identify the 2-MG band. Sample was scraped and eluted with chloroform. The fatty acid methyl ester of 2-MG were prepared using boron triflouride as mentioned before.

### H. Statistical Analysis

The results were expressed as means and standard deviations of two replications. Palmitic acid enrichment factors and lard substitution levels were analysed to obtain the trend line equation as well as R2 value using the Microsoft excel software.

## III. Results and Discussion

From the large of effort that has already been expanded on the triglyceride structure of lard, it is evident that lard contains mainly palmitic acid in 2-MG. Luddy et al. [20] analyzed lard by lipase hydrolysis and found that about 83% of palmitic acid, 10-20% oleic acid and 2-12% stearic acid are esterifies in the 2-MG. Pancreatic lipolysis method was used to know how the distribution of esterified acids in triglycerides is. In most cases, the position 2-MG contains mainly unsaturated fatty acids. The principal exception of this distribution was reported in lard which contains higher levels of palmitic acid in this position [16].

The fatty acid composition of the TGs and 2-MG obtained by pancreatic lipolysis of camel, beef, sheep, goat, rabbit, chicken mixtures and genuine lard are shown in Tables I-VI. In the case of genuine lard a high proportion (60.97 %) of the total palmitic acid is esterifies at the position 2-MG. This accordance with the data cited in the literature [21]. On the

other hand, the 2-monopalmitin content in the case of camel fat was lower (8.70%) as shown in Table I. Mattson et al. [4] reported that camel fat contains 9 % palmitic acid at 2-MG. Results indicated also the position 2-MG is mostly occupied by unsaturated fatty acids in camel fat (82.32%). Moreover, palmitic acid content in 2-MG increased gradually as the levels of lard increased in camel mixtures as shown in Table I. Palmitic acid enrichment factor (PAEF) of the investigated samples increased as the substitution levels increased with lard up to 20 %. The increases in PAEF in camel mixtures ranged from 0.25 (0 % lard) to 0.61 (20% lard).

TABLE I
FATTY ACID COMPOSITION IN TRIACYLGLYCEROL (TG), 2-MONOACYL- GLYCEROL (2-MG) AND PALMITIC ACID ENRICHMENT FACTOR (PAEF) OF CAMEL FAT SUBSTITUTED WITH DIFFERENT LEVELS OF LARD

| Lard (%) | | Fatty acid (%) | | | | | | | *PAEF |
|---|---|---|---|---|---|---|---|---|---|
| | | C14:0 | C16:0 | C16:1 | C18:0 | C 18:1 | C18:2 | C18:3 | |
| 0 | TG | 7.13±0.02 | 34.92±0.02 | 1.33±0.01 | 34.71±0.06 | 21.93±0.06 | ND | ND | 0.25 |
| | 2-MG | 1.90±0.14 | 8.70±0.71 | 5.58±0.20 | 7.08±0.12 | 71.48±0.08 | 5.26±0.19 | ND | |
| 1 | TG | 6.19±0.33 | 35.53±0.12 | 2.63±0.04 | 31.84 ±0.50 | 23.83 ±0.10 | ND | ND | 0.26 |
| | 2-MG | 1.37±0.03 | 9.05±0.07 | 4.88±0.08 | 6.89±0.78 | 72.81±0.04 | 5.00±0.08 | ND | |
| 5 | TG | 4.16±0.01 | 32.41±0.01 | 2.49±0.02 | 38.11±0.01 | 22.84±0.01 | ND | ND | 0.38 |
| | 2-MG | 2.14±0.04 | 12.28±0.05 | 5.02±0.06 | 7.61±0.02 | 68.15±0.11 | 4.80±0.03 | ND | |
| 10 | TG | 4.17±0.01 | 31.91±0.08 | 2.78±0.03 | 37.74±0.01 | 23.41±0.12 | ND | ND | 0.45 |
| | 2-MG | 1.46±0.25 | 14.37±0.08 | 5.42±0.37 | 6.57±0.21 | 67.04±0.44 | 5.14±0.11 | ND | |
| 20 | TG | 3.91±0.01 | 31.31±0.11 | 2.42±0.03 | 40.22±0.05 | 22.15±0.14 | ND | ND | 0.61 |
| | 2-MG | 2.94±0.233 | 19.19±0.21 | 5.29±0.02 | 6.93±0.32 | 59.77±0.25 | 5.88±0.11 | ND | |
| Lard | TG | 1.34±0.01 | 24.05±0.11 | 4.45±0. 10 | 15.48±0.02 | 37.89±0.12 | 15.53±0.07 | 1.28±0.01 | 2.54 |
| | 2-MG | 3.53±0.67 | 60.97±0.52 | ND | 9.73±0.52 | 16.74±0.76 | 9.03±0.96 | ND | |

* PAEF= Palmitic acid (%) in 2-MG/Palmitic acid (%) in TG.

Table II showed that beef fat contain 16.40% palmitic acid in 2-MG and increased gradually as the substitution levels of lard increase. These results are confirmed with that obtained by [11], [1] who revealed that beef fat contains 16.0 – 16.7% palmitic in 2-MG, while [22] found that beef fat contains 12% palmitic acid in 2-MG. Results indicated also the position-2-MG is mostly occupied by unsaturated fatty acids being 67.53%, while it was lower in lard (35.50%).

Sheep fat contain 11.38% palmitic acid esterifies at 2-MG position and 73.87 % unsaturated fatty acids in the same position as shown in Table III. Christie and Moore [21] and Mattson et al. [4] found that sheep fat contains 9.11% palmitic acid in 2-MG. However, Abou-Hadeed et al. [22] and Al-Rashood et al. [11] reported 14% pamitic acid in 2-MG.

TABLE II
FATTY ACID COMPOSITION IN TRIACYLGLYCEROL (TG), 2-MONOACYL- GLYCEROL (2-MG) AND PALMITIC ACID ENRICHMENT FACTOR (PAEF) OF BEEF FAT SUBSTITUTED WITH DIFFERENT LEVELS OF LARD

| Lard (%) | | Fatty acid (%) | | | | | | | *PAEF |
|---|---|---|---|---|---|---|---|---|---|
| | | C14:0 | C16:0 | C16:1 | C18:0 | C18:1 | C18:2 | C18:3 | |
| 0 | TG | 5.02±0.02 | 35.74±0.02 | 1.60±0.03 | 35.09±0.01 | 22.56±0.01 | ND | ND | 0.46 |
| | 2-MG | 7.16±1.39 | 16.40±0.26 | 6.38±0.42 | 8.91±0.05 | 53.31±0.78 | 6.86±0.98 | 0.98±0.18 | |
| 1 | TG | 5.31±0.01 | 32.22±0.04 | 2.28±0.02 | 39.63±0.02 | 20.57±0.02 | ND | ND | 0.54 |
| | 2-MG | 5.75±0.18 | 17.25±0.13 | 5.64±0.01 | 10.12±1.08 | 53.40±1.64 | 6.90±0.90 | 0.90±0.02 | |
| 5 | TG | 5.50±0.15 | 30.06±0.05 | 2.68±0.12 | 41.43±0.25 | 20.34±0.46 | ND | ND | 0.79 |
| | 2-MG | 5.43±0.19 | 23.66±0.86 | 5.67±0.66 | 10.22±0.27 | 46.47±0.66 | 7.40±0.53 | 1.15±0.26 | |
| 10 | TG | 2.91±0.67 | 30.59±0.40 | 2.56±0.04 | 43.74±0.50 | 20.21±0.61 | ND | ND | 0.84 |
| | 2-MG | 5.50±0.20 | 25.71±0.53 | 5.24±0.12 | 9.47±0.60 | 46.04±0.09 | 6.92±0.06 | 1.12±0.28 | |
| 20 | TG | 4.44±0.44 | 29.49±0.42 | 2.15±0.05 | 44.56±0.44 | 19.36±0.37 | ND | ND | 1.15 |
| | 2-MG | 5.04±0.47 | 33.96±0.10 | 3.33±0.02 | 10.76±0.24 | 39.29±0.12 | 5.76±0.36 | 1.86±0.08 | |
| Lard | TG | 1.34±0.01 | 24.05±0.11 | 4.45±0.10 | 15.48±0.02 | 37.89±0.12 | 15.53±0.07 | 1.28±0.01 | 2.54 |
| | 2-MG | 3.53±0.67 | 60.97±0.523 | ND | 9.73±0.52 | 16.74±0.76 | 9.03±0.96 | ND | |

*PAEF= Palmitic acid (%) in 2-MG/Palmitic acid (%) in TG

Data in Table IV showed that 10.57% palmitic acid and 65.65% unsaturated fatty acids are found in 2-MG of goat fat. Abou-Hadeed et al. [22] reported that the levels of palmitic acid in 2-MG of goat was 12.67%.

The levels of palmitic acid in rabbit and chicken fats in 2-MG were 29.97% and 8.97 %, and unsaturated fatty acids being 53.77 and 81.14 %, respectively as shown in Tables V and VI. This results are agree with the results obtained by [21] who found that rabbit fat contains 25% palmitic acid in 2-MG and chicken fat contains 13% in the same position.

The obtained results exhibited that palmitic acid esterifies at 2-MG increased gradually as the substituted levels increased among all tested fat samples under investigation. Moreover, rabbit fat contained a higher levels of palmitic acid in 2-MG (29.97%) followed by beef fat (16.40%), then sheep, goat, chicken and camel fats being 11.38, 10.57, 8.97 and 8.7%, respectively.

Open Science Index, Nutrition and Food Engineering Vol:8, No:11, 2014 publications.waset.org/10000133/pdf

TABLE III
FATTY ACID COMPOSITION IN TRIACYLGLYCEROL (TG), 2-MONOACYL- GLYCEROL (2-MG) AND PALMITIC ACID ENRICHMENT FACTOR (PAEF) OF SHEEP FAT SUBSTITUTED WITH DIFFERENT LEVELS OF LARD

| Lard (%) | | Fatty acid (%) | | | | | | | *PAEF |
|---|---|---|---|---|---|---|---|---|---|
| | | C14:0 | C16:0 | C16:1 | C18:0 | C18:1 | C18:2 | C18:3 | |
| 0 | TG | 8.50±0.25 | 20.75±0.2 | 3.97±0.04 | 35.12±0.01 | 23.69±0.42 | 5.00±0.02 | 2.97±0.01 | 0.55 |
| | 2-MG | 3.49±0.15 | 11.38±0.26 | 3.61±0.09 | 11.26±0.65 | 63.21±0.15 | 5.98±0.10 | 1.07±0.10 | |
| 1 | TG | 4.10±0.20 | 24.32±0.21 | 2.95±0.46 | 40.12±0.48 | 22.50±0.18 | 4.21±0.03 | 1.80±0.20 | 0.56 |
| | 2-MG | 3.30±0.35 | 13.55±0.42 | 3.28±0.07 | 11.66±0.18 | 59.07±0.47 | 8.39±0.58 | 0.75±0.10 | |
| 5 | TG | 6.02±0.90 | 19.16±0.22 | 3.29±0.27 | 41.95±1.67 | 22.40±0.38 | 3.70±0.62 | 3.48±0.48 | 0.79 |
| | 2-MG | 3.50±0.042 | 15.17±0.05 | 2.42±0.01 | 11.33±0.13 | 60.32±0.33 | 6.16±0.06 | 1.10±0.23 | |
| 10 | TG | 3.98±0.02 | 22.51±0.15 | 3.84±0.33 | 44.90±0.02 | 18.66±0.06 | 3.55±0.03 | 2.56±0.01 | 0.85 |
| | 2-MG | 3.45±0.11 | 19.11±0.08 | 2.42±0.04 | 9.41±0.12 | 59.03±0.23 | 5.50±0.59 | 1.08±0.16 | |
| 20 | TG | 3.00±0.01 | 23.62±0.11 | 2.64±0.19 | 43.69±0.22 | 22.30±0.30 | 3.31±0.33 | 1.44±0.3 | 0.93 |
| | 2-MG | 3.14±0.02 | 21.98±0.14 | 2.73±0.07 | 9.66±0.08 | 55.50±0.50 | 5.96±0.37 | 1.03±0.04 | |
| Lard | TG | 1.34±0.01 | 24.05±0.11 | 4.45±0.10 | 15.48±0.02 | 37.89±0.12 | 15.53±0.07 | 1.28±0.01 | 2.54 |
| | 2-MG | 3.53±0.67 | 60.97±0.52 | ND | 9.73±0.52 | 16.74±0.76 | 9.03±0.96 | ND | |

*PAEF= Palmitic acid (%) in 2-MG/Palmitic acid (%) in TG

TABLE IV
FATTY ACID COMPOSITION IN TRIACYLGLYCEROL (TG), 2-MONOACYL- GLYCEROL (2-MG) AND PALMITIC ACID ENRICHMENT FACTOR (PAEF) OF GOAT FAT SUBSTITUTED WITH DIFFERENT LEVELS OF LARD

| Lard (%) | | Fatty acid (%) | | | | | | | *PAEF |
|---|---|---|---|---|---|---|---|---|---|
| | | C14:0 | C16:0 | C16:1 | C18:0 | C18:1 | C18:2 | C18:3 | |
| 0 | TG | 3.26±0.05 | 21.98±0.02 | 3.60±0.07 | 24.66±0.14 | 40.44±0.03 | 6.06±0.06 | ND | 0.48 |
| | 2-MG | 3.37±0.11 | 10.57±0.21 | 2.13±0.36 | 20.41±0.29 | 59.16±0.41 | 4.36±0.02 | ND | |
| 1 | TG | 3.58±0.31 | 19.53±0.17 | 4.05±0.02 | 29.74±0.27 | 37.80±0.19 | 5.30±0.30 | ND | 0.76 |
| | 2-MG | 4.37±0.46 | 14.88±0.88 | 2.38±0.27 | 20.34±0.69 | 51.30±0.35 | 6.73±0.35 | ND | |
| 5 | TG | 2.84±0.17 | 7.28±0.841 | 4.09±0.17 | 29.78±0.28 | 40.78±0.78 | 5.23±0.34 | ND | 1.17 |
| | 2-MG | 4.57±0.49 | 20.29±0.21 | 2.82±0.50 | 18.47±0.04 | 47.47±0.41 | 6.38±0.03 | ND | |
| 10 | TG | 4.16±0.02 | 16.89±0.11 | 4.33±0.11 | 28.43±0.09 | 39.93±0.26 | 6.26±0.03 | ND | 1.63 |
| | 2-MG | 5.25±0.601 | 27.57±0.23 | 2.57±0.10 | 11.62±0.02 | 46.62±0.06 | 6.37±0.34 | ND | |
| 20 | TG | 3.46±0.46 | 18.07±0.91 | 3.65±0.21 | 28.86±0.32 | 40.39±0.51 | 5.57±0.04 | ND | 1.89 |
| | 2-MG | 2.68±0.33 | 34.15±0.41 | 2.52±0.84 | 10.23±0.52 | 41.03±1.17 | 6.39±0.22 | ND | |
| Lard | TG | 1.34±0.01 | 24.05±0.11 | 4.45±0.10 | 15.48±0.02 | 37.89±0.12 | 15.53±0.07 | 1.28±0.01 | 2.54 |
| | 2-MG | 3.53±0.67 | 60.97±0.52 | ND | 9.73±0.52 | 16.74±0.76 | 9.03±0.96 | ND | |

*PAEF= Palmitic acid (%) in 2-MG/Palmitic acid (%) in TG

TABLE V
FATTY ACID COMPOSITION IN TRIACYLGLYCEROL (TG), 2-MONOACYL- GLYCEROL (2- MG) AND PALMITIC ACID ENRICHMENT FACTOR (PAEF) OF RABBIT FAT SUBSTITUTED WITH DIFFERENT LEVELS OF LARD

| Lard (%) | | Fatty acid (%) | | | | | | | *PAEF |
|---|---|---|---|---|---|---|---|---|---|
| | | C14:0 | C16:0 | C16:1 | C18:0 | C18:1 | C18:2 | C18:3 | |
| 0 | TG | 2.16±0.01 | 26.20±0.05 | 1.03±0.08 | 19.09±0.16 | 36.15±0.28 | 13.87±0.49 | 1.50±0.14 | 1.14 |
| | 2-MG | 4.79±0.13 | 29.97±0.03 | 7.67±0.93 | 11.47±0.19 | 28.99±1.10 | 15.42±0.92 | 1.69±0.25 | |
| 1 | TG | 3.64±0.35 | 24.04±0.03 | 1.60±0.3 | 18.70±0.07 | 38.95±0.05 | 13.07±0.44 | 1.22±0.08 | 1.35 |
| | 2-MG | 5.11±0.82 | 32.34±0.17 | 6.58±0.56 | 11.15±0.14 | 28.52±0.31 | 15.12±0.12 | 1.18±0.01 | |
| 5 | TG | 2.45±0.02 | 22.47±0.01 | 1.82±0.02 | 18.51±0.10 | 41.30±0.24 | 12.21±0.01 | 1.24±0.15 | 1.58 |
| | 2-MG | 4.02±0.36 | 35.38±0.46 | 5.97±0.16 | 9.39±0.13 | 29.18±0.03 | 15.01±0.47 | 1.05±0.03 | |
| 10 | TG | 2.60±0.27 | 21.72±0.12 | 1.48±0.01 | 19.28±0.14 | 41.60±0.68 | 11.71±0.56 | 1.61±0.14 | 1.69 |
| | 2-MG | 4.55±0.21 | 36.62±0.56 | 4.86±0.09 | 11.67±0.10 | 30.72±0.16 | 10.54±0.05 | 1.04±0.03 | |
| 20 | TG | 2.07±0.01 | 20.62±0.02 | 1.80±0.01 | 18.25±0.27 | 42.92±0.73 | 12.33±0.50 | 2.01±0.01 | 1.79 |
| | 2-MG | 4.18±0.11 | 36.88±0.26 | 4.88±0.06 | 10.31±0.04 | 31.06±0.04 | 11.34±0.45 | 1.35±0.07 | |
| Lard | TG | 1.34±0.01 | 24.05±0.11 | 4.45±0.10 | 15.48±0.02 | 37.89±0.12 | 15.53±0.07 | 1.28±0.01 | 2.54 |
| | 2-MG | 3.53±0.67 | 60.97±0.52 | ND | 9.73±0.52 | 16.74±0.76 | 9.03±0.961 | ND | |

*PAEF= Palmitic acid (%) in 2-MG/Palmitic acid (%) in TG

TABLE VI
FATTY ACID COMPOSITION IN TRIACYLGLYCEROL (TG), 2-MONOACYL- GLYCEROL (2-MG) AND PALMITIC ACID ENRICHMENT FACTOR (PAEF) OF CHICKEN FAT SUBSTITUTED WITH DIFFERENT LEVELS OF LARD

| Lard (%) | | Fatty acid (%) | | | | | | | *PAEF |
|---|---|---|---|---|---|---|---|---|---|
| | | C14:0 | C16:0 | C16:1 | C18:0 | C18:1 | C18:2 | C18:3 | |
| 0 | TG | 1.13±0.18 | 21.25±0.05 | 5.64±0.04 | 12.92±0.31 | 47.74±0.47 | 10.42±0.01 | 0.90±0.01 | 0.42 |
| | 2-MG | 2.35±0.17 | 8.97±0.12 | 3.85±0.01 | 7.54±0.33 | 49.40±0.17 | 27.89±0.12 | ND | |
| 1 | TG | 1.78±0.01 | 17.86±0.03 | 3.58±0.01 | 20.44±0.02 | 47.53±0.53 | 7.30±0.28 | 1.51±0.19 | 0.54 |
| | 2-MG | 0.67±0.035 | 9.69±0.13 | 3.16±0.11 | 4.29±0.28 | 56.08±0.46 | 26.11±0.10 | ND | |
| 5 | TG | 1.58±0.23 | 7.54±0.055 | 3.63±0.19 | 24.50±0.48 | 45.29±0.29 | 6.16±0.01 | 1.30±0.22 | 0.91 |
| | 2-MG | 0.77±0.07 | 16.01±0.22 | 4.13±0.16 | 4.81±0.23 | 49.08±0.96 | 25.20±0.30 | ND | |
| 10 | TG | 1.08±0.04 | 18.24±0.01 | 3.61±0.04 | 22.28±0.07 | 47.40±0.18 | 6.10±0.03 | 1.29±0.02 | 1.04 |
| | 2-MG | 1.21±0.01 | 18.95±0.26 | 4.39±0.02 | 5.40±0.03 | 47.17±0.45 | 22.88±0.15 | ND | |
| 20 | TG | 1.60±0.13 | 17.70±0.22 | 2.69±0.69 | 21.48±0.52 | 47.51±0.03 | 2.21±0.01 | 6.81±0.05 | 1.51 |
| | 2-MG | 2.33±0.03 | 26.74±0.24 | 4.63±0.01 | 8.07±0.42 | 42.53±0.04 | 15.70±0.18 | ND | |
| Lard | TG | 1.34±0.01 | 24.05±0.11 | 4.45±0.10 | 15.48±0.02 | 37.89±0.12 | 15.53±0.07 | 1.28±0.01 | 2.54 |
| | 2-MG | 3.53±0.67 | 60.97±0.523 | ND | 9.73±0.52 | 16.74±0.76 | 9.03±0.96 | ND | |

*PAEF= Palmitic acid (%) in 2-MG/Palmitic acid (%) in TG

Open Science Index, Nutrition and Food Engineering Vol:8, No:11, 2014 publications.waset.org/10000133/pdf

With respect to the vegetable oil mixtures (corn, sunflower, palm oil and olive oil) under investigation, it could be noticed that the levels of palmitic acid esterifies at 2-MG position of corn, sunflower, palm oil and olive oil were 6.84, 1.43, 9.86 and 1.70%, respectively. It could be observed also the studied oils had a higher level of unsaturated fatty acids in the same position, compared with animal fats under investigation as shown in Tables VII-X. These results are in good agreement with that obtained by [23], [24]. Moreover, palmitic acid content increased gradually as the levels of lard increase among all the tested oil mixtures.

Palmitic acid enrichment factors in this study increased gradually as the substitution levels increase with different levels among all tested samples either in fats or oils (Figs. 1 and 2).

TABLE VII
FATTY ACID COMPOSITION IN TRIACYLGLYCEROL (TG), 2-MONOACYL- GLYCEROL (2-MG) AND PALMITIC ACID ENRICHMENT FACTOR (PAEF) OF CORN OIL SUBSTITUTED WITH DIFFERENT LEVELS OF LARD

| Lard (%) | | Fatty acid (%) | | | | | | | *PAEF |
|---|---|---|---|---|---|---|---|---|---|
| | | C14:0 | C16:0 | C16:1 | C18:0 | C18:1 | C18:2 | C18:3 | |
| 0 | TG | ND | 14.17±0.12 | ND | 3.28±0.01 | 28.46±0.12 | 54.09±0.01 | ND | 0.48 |
| | 2-MG | ND | 6.84±0.50 | ND | 3.49±0.83 | 24.14±1.27 | 65.53±1.90 | ND | |
| 1 | TG | ND | 14.14±0.11 | ND | 3.46 ±0.01 | 27.90 ±0.74 | 54.50±0.64 | ND | 0.88 |
| | 2-MG | ND | 12.52±0.01 | ND | 3.12±0.11 | 26.29±0.11 | 58.07±0.22 | ND | |
| 5 | TG | ND | 14.42±0.21 | ND | 4.01±0.07 | 28.68±0.06 | 52.89±0.20 | ND | 0.91 |
| | 2-MG | ND | 13.15±0.50 | ND | 3.69±0.17 | 24.02±0.62 | 59.14±1.30 | ND | |
| 10 | TG | ND | 14.73±0.09 | ND | 4.22±0.01 | 29.32±0.06 | 51.73±0.01 | ND | 0.95 |
| | 2-MG | ND | 14.06±0.18 | ND | 2.74±0.18 | 24.74±0.28 | 58.46±0.28 | ND | |
| 20 | TG | ND | 14.38±0.23 | ND | 4.28±0.13 | 30.59±0.15 | 50.75±0.05 | ND | 1.23 |
| | 2-MG | ND | 17.73±0.14 | ND | 3.44±0.15 | 23.88±0.35 | 54.95±0.70 | ND | |
| Lard | TG | 1.34±0.01 | 24.05±0.11 | 4.45±0.10 | 15.48±0.02 | 37.89±0.12 | 15.53±0.07 | 1.28±0.01 | 2.54 |
| | 2-MG | 3.53±0.67 | 60.97±0.52 | ND | 9.73±0.52 | 16.74±0.76 | 9.03±0.96 | ND | |

*PAEF= Palmitic acid (%) in 2-MG/Palmitic acid (%) in TG

TABLE VIII
FATTY ACID COMPOSITION IN TRIACYLGLYCEROL (TG), 2-MONOACYL- GLYCEROL (2-MG) AND PALMITIC ACID ENRICHMENT FACTOR (PAEF) OF SUNFLOWER OIL SUBSTITUTED WITH DIFFERENT LEVELS OF LARD

| Lard (%) | | Fatty acid (%) | | | | | | | *PAEF |
|---|---|---|---|---|---|---|---|---|---|
| | | C14:0 | C16:0 | C16:1 | C18:0 | C18:1 | C18:2 | C18:3 | |
| 0 | TG | ND | 5.08±0.05 | ND | 4.17±0.08 | 29.20±0.04 | 61.55±0.17 | ND | 0.28 |
| | 2-MG | ND | 1.43±0.01 | ND | 0.55±0.01 | 28.66±0.15 | 69.36±0.04 | ND | |
| 1 | TG | ND | 5.25±0.15 | ND | 4.27±0.08 | 29.60±0.08 | 60.88±0.01 | ND | 0.59 |
| | 2-MG | ND | 3.10±0.01 | ND | 1.10±0.01 | 27.66±0.01 | 68.14±0.06 | ND | |
| 5 | TG | ND | 5.95±0.23 | ND | 4.58±0.25 | 29.73±0.19 | 59.74±0.22 | ND | 0.74 |
| | 2-MG | ND | 4.39±0.01 | ND | 1.45±0.01 | 27.46±0.01 | 66.70±0.01 | ND | |
| 10 | TG | ND | 6.25±0.06 | ND | 5.00±0.13 | 30.90±0.04 | 57.85±0.11 | ND | 1.02 |
| | 2-MG | ND | 6.37±0.04 | ND | 1.27±0.01 | 25.98±0.01 | 66.38±0.08 | ND | |
| 20 | TG | ND | 7.84±0.25 | ND | 6.70±0.83 | 30.23±0.26 | 55.23±0.82 | ND | 1.72 |
| | 2-MG | ND | 13.52±0.03 | ND | 2.00±0.08 | 23.34±0.01 | 61.14±0.04 | ND | |
| Lard | TG | 1.34±0.005 | 24.05±0.11 | 4.45±0.10 | 15.48±0.02 | 37.89±0.12 | 15.53±0.07 | 1.28±0.01 | 2.54 |
| | 2-MG | 3.53±0.671 | 60.97±0.52 | ND | 9.73±0.52 | 16.74±0.76 | 9.03±0.96 | ND | |

*PAEF= Palmitic acid (%) in 2-MG/Palmitic acid (%) in TG

TABLE IX
FATTY ACID COMPOSITION IN TRIACYLGLYCEROL (TG), 2-MONOACYL- GLYCEROL (2-MG) AND PALMITIC ACID ENRICHMENT FACTOR (PAEF) OF PALM OIL SUBSTITUTED WITH DIFFERENT LEVELS OF LARD

| Lard (%) | | Fatty acid (%) | | | | | | | *PAEF |
|---|---|---|---|---|---|---|---|---|---|
| | | C14:0 | C16:0 | C16:1 | C18:0 | C18:1 | C18:2 | C18:3 | |
| 0 | TG | ND | 36.47±0.52 | ND | 5.16±0.14 | 46.88±0.98 | 11.49±0.23 | ND | 0.27 |
| | 2-MG | ND | 9.86±0.04 | ND | 0.78±0.01 | 65.20±0.02 | 24.16±0.03 | ND | |
| 1 | TG | ND | 36.55±0.43 | ND | 5.96±0.02 | 46.10±0.18 | 11.39±0.03 | ND | 0.31 |
| | 2-MG | ND | 11.45±0.01 | ND | 1.12±0.12 | 63.76±0.17 | 23.67±0.04 | ND | |
| 5 | TG | ND | 37.38±0.98 | ND | 5.30±0.04 | 45.91±0.26 | 11.41±0.06 | ND | 0.37 |
| | 2-MG | ND | 13.93±0.01 | ND | 2.90±0.43 | 59.77±0.20 | 23.40±0.03 | ND | |
| 10 | TG | ND | 38.41±0.23 | ND | 5.76±0.07 | 43.98±0.37 | 11.85±0.08 | ND | 0.57 |
| | 2-MG | ND | 21.86±0.21 | ND | 1.04±0.01 | 54.20±0.01 | 22.90±0.02 | ND | |
| 20 | TG | ND | 39.10±0.36 | ND | 6.73±0.13 | 42.67±0.69 | 11.50±0.17 | ND | 0.58 |
| | 2-MG | ND | 22.50±0.01 | ND | 2.36±0.01 | 53.76±0.09 | 21.38±0.09 | ND | |
| Lard | TG | 1.34±0.01 | 24.05±0.11 | 4.45±0.10 | 15.48±0.02 | 37.89±0.12 | 15.53±0.07 | 1.28±0.01 | 2.54 |
| | 2-MG | 3.53±0.67 | 60.97±0.52 | ND | 9.73±0.52 | 16.74±0.76 | 9.03±0.96 | ND | |

PAEF= Palmitic acid (%) in 2-MG/Palmitic acid (%) in TG

Open Science Index, Nutrition and Food Engineering Vol:8, No:11, 2014 publications.waset.org/10000133/pdf

TABLE X
FATTY ACID COMPOSITION IN TRIACYLGLYCEROL (TG), 2-MONOACYL- GLYCEROL (2-MG) AND PALMITIC ACID ENRICHMENT FACTOR (PAEF) OF OLIVE OIL SUBSTITUTED WITH DIFFERENT LEVELS OF LARD

| Lard (%) | | Fatty acid (%) | | | | | | | *PAEF |
|---|---|---|---|---|---|---|---|---|---|
| | | C14:0 | C16:0 | C16:1 | C18:0 | C18:1 | C18:2 | C18:3 | |
| 0 | TG | ND | 9.65±0.18 | ND | 3.19±0.02 | 79.21±0.15 | 7.35±0.01 | 0.60±0.01 | 0.18 |
| | 2-MG | ND | 1.70±0.42 | ND | 5.08±0.36 | 81.13±1.02 | 9.80±0.91 | 2.29±0.89 | |
| 1 | TG | ND | 9.46±0.26 | ND | 3.25±0.01 | 79.23±0.28 | 7.44±0.01 | 0.62±0.01 | 0.27 |
| | 2-MG | ND | 2.56±0.16 | ND | 3.84±0.61 | 77.54±0.53 | 13.10±0.21 | 2.96±0.44 | |
| 5 | TG | ND | 9.88±0.15 | ND | 3.60±0.02 | 78.34±0.12 | 7.48±0.21 | 0.70±0.01 | 0.56 |
| | 2-MG | ND | 5.56±0.07 | ND | 3.28±0.08 | 74.22±0.33 | 15.49±0.01 | 1.45±0.18 | |
| 10 | TG | ND | 10.41±0.11 | ND | 4.01±0.01 | 76.90±0.08 | 8.11±0.01 | 0.57±0.01 | 0.98 |
| | 2-MG | ND | 10.23±0.13 | ND | 4.52±0.01 | 66.51±0.15 | 16.54±0.26 | 2.20±0.240 | |
| 20 | TG | ND | 10.95±0.28 | ND | 4.78±0.03 | 74.45±0.23 | 9.23±0.03 | 0.59±0.01 | 1.29 |
| | 2-MG | ND | 14.15±0.28 | ND | 9.11±1.36 | 57.20±0.29 | 18.00±0.784 | 1.54±0.02 | |
| Lard | TG | 1.34±0.01 | 24.05±0.11 | 4.45±0.10 | 15.48±0.02 | 37.89±0.12 | 15.53±0.07 | 1.28±0.01 | 2.54 |
| | 2-MG | 3.53±0.67 | 60.97±0.52 | ND | 9.73±0.52 | 16.74±0.76 | 9.03±0.96 | ND | |

*PAEF= Palmitic acid (%) in 2-MG/Palmitic acid (%) in TG

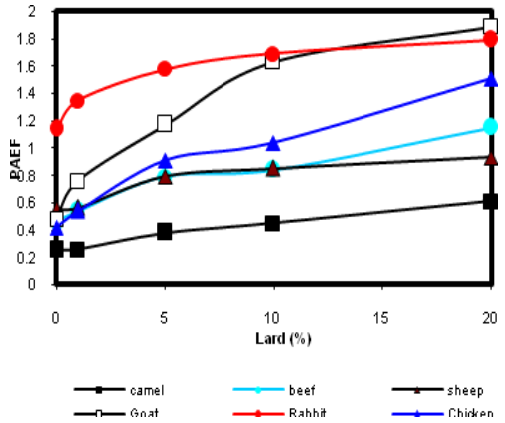



Fig. 1 Relationship between lard levels and palmitic acid enrichment factor (PAEF) of animal fats

Statistical analysis showed that the PAEF correlated well with lard (%) as shown in Table XI. Correlation coefficient could be arranged in the following descending order: camel ($R^2$=0.9828), sunflower oil ($R^2$=0.9736), chicken ($R^2$=0.9602), olive oil ($R^2$= 0.9526), beef fat ($R^2$= 0.9472), goat fat ($R^2$= 0.8859), palm oil ($R^2$= 0.8286), sheep fat ($R^2$= 0.8229), and rabbit fat ($R^2$= 0.7761).

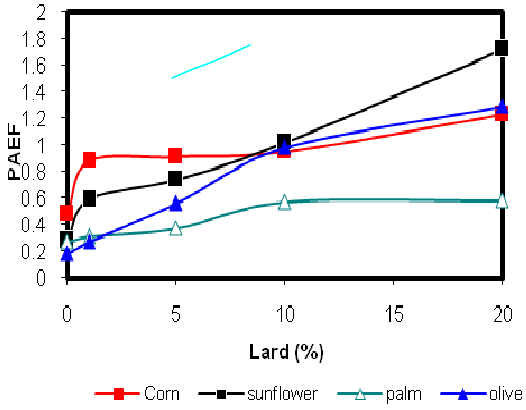



Fig. 2 Relationship between lard levels and palmitic acid enrichment factor (PAEF) of vegetable oils

### *A. Prediction of Lard in Processed Food Products from Local Market*

The proposed scheme was adopted to check the presence or absence of lard in a wide variety of processed foods collected from Riyadh local markets. The processed food samples used in this study include 5 French fries, 4 Butter fat (3 animal butter and 1 vegetable butter), 5 processed meat and 6 candy (chocolate) samples. Fats from collected samples were, extracted separately by [25] for meat samples, hexane for French fries and candy. Butter fat was melted by heating in air oven at the melting point (±5°C). Triglycerides and 2-MG were separated using benzene and pancreatic lipolysis, respectively. The fatty acid methyl esters of each fraction were prepared using boron triflouride, and then analyzed by gas chromatography (GC) by adopting the specific separation conditions.

TABLE XI
CORRELATION BETWEEN PALMITIC ACID ENRICHMENT FACTOR AND SUBSTITUTION LEVELS OF LARD

| No | Fats/Oils | Equation | $R^2$ |
|---|---|---|---|
| 1 | Camel | Y=0.0181x + 0.2599 | 0.9828 |
| 2 | Beef | Y=0.0325x + 0.5219 | 0.9472 |
| 3 | Sheep | Y=0.0192x + 0.5980 | 0.8229 |
| 4 | Goat | Y=0.0675x + 0.6999 | 0.8859 |
| 5 | Rabbit | Y=0.0284x + 1.3052 | 0.7761 |
| 6 | Chicken | Y=0.0520x + 0.5098 | 0.9602 |
| 7 | Corn | Y=0.0281x + 0.6879 | 0.7306 |
| 8 | Sunflower | Y=0.0659x + 0.3958 | 0.9736 |
| 9 | Palm | Y=0.0163x + 0.3029 | 0.8286 |
| 10 | Olive | Y=0.0564x + 0.2497 | 0.9526 |
| | | Y: PAEF | x : Lard % |

For the detection of lard, it's not possible to specify the other fat in the mixture, until we know the sample specification i.e. label on the sample. After knowing the numbers of fats or oils used for the preparation of mixtures, comparison of fatty acid of the mixtures to that of genuine fats or oils were carried out.

### *B. French Fries*

Fatty acids in TG and 2-MG as well as PAEF of fat extracted from five samples of French fries collected from local market are shown in Table XII. It is clear that 2-MG had lower levels of C16:0 as compared to TG for samples 1, 2, 3 and 5 being 8.24, 9.02, 3.75, 7.74%, respectively. However,

Open Science Index, Nutrition and Food Engineering Vol:8, No:11, 2014 publications.waset.org/10000133/pdf

sample 4 had a higher level of C16:0 at 2-MG, compared to its TG being 10.20 and 9.97%., respectively. Calculation of Palmitic acid enrichment factors (PAEFs) and linear regression equation (LRE) No. 9 of tested samples indicated that, samples 1, 2, and 3 had a lower values being 0.24, 0.25 and 0.28, respectively. This indicated that these three samples are free of lard. Moreover, comparing the fatty acid composition in TG and 2-MG as well as PAEFs revealed that these samples were fried in palm oil as indicated by the label on the products. The higher values of PAEFs of samples 4 and 5 (1.20 and 0.74, respectively) may contain lard fat or other mixed fat or oils as indicated on its labels. Sample 4 fried in mixed oils (Canola and soybean oils as reported on its label). Comparing the fatty acid in TG and 2-MG (Table XII) with that reported previously, it could be noticed that both soybean and canola oils contains 1 % of palmitic acid in 2-MG, while it was 3 and 9% in TG of canola and soybean oils, respectively as reported by [26]. Sample 5 also contains more than one fat or oil as reported on its label (sunflower, cotton, soybean, palm oil, canola and partially hydrogenated soybean oil). From previous results as mentioned by [26], [27], cotton seed, soybean and canola oils contains 2, 1 and 1% palmitic acid in 2-MG, respectively. Thus, comparing with our results, it could suggest that sample 5 may be contains lard. Linear regression equation could not be used in both samples (4 and 5) because they contain more than one fat or oil. From these results it could be concluded that samples 1, 2 and 3 did not contains lard, while samples 4 and 5 contained lard due to their higher PAEF.

TABLE XII
FATTY ACID COMPOSITION IN TRIACYLGLYCEROL (TG), 2-MONOACYL- GLYCEROL (2-MG) AND PALMITIC ACID ENRICHMENT FACTOR (PAEF) OF SOME FRENCH FRIES COLLECTED FROM LOCAL MARKET

| No | Coded Samples | | Fatty acids (%) | | | | | | *PAEF |
|---|---|---|---|---|---|---|---|---|---|
| | | | C16:0 | C16:1 | C18:0 | C18:1 | C18:2 | 18:3 | |
| 1 | Frozen French Fries Pre-Fried in Palm Oil (Holland) | TG | 34.74±0.19 | - | 4.97±0.08 | 48.60±0.43 | 11.69±0.83 | - | 0.24 |
| | | 2-MG | 8.24±0.61 | - | 2.48±0.15 | 61.63±0.14 | 27.65±0.57 | - | |
| 2 | Frozen French Fries Pre-Fried in Palm Oil (Holland) | TG | 35.40±0.44 | - | 5.24±0.28 | 47.23±0.33 | 12.13±0.14 | - | 0.25 |
| | | 2-MG | 9.02±0.02 | - | 3.42±0.55 | 61.38±0.55 | 26.18±0.42 | - | |
| 3 | Frozen French Fries Pre-Fried in Sunflower, Cotton seed, Soybean and Canola oils (USA) Frozen French Fries Pre-Fried in Soybean and Canola oils (Canada) | TG | 13.19±0.17 | 0.37±0.02 | 1.21±0.01 | 44.76±0.04 | 40.47±0.04 | - | 0.28 |
| | | 2-MG | 3.75±0.20 | - | - | 49.59±0.72 | 46.67±0.52 | - | |
| 4 | Frozen French Fries Pre-Fried in Sunflower, Cotton seed, Soybean, Palm, Canola and | TG | 9.97±0.14 | - | 5.67±0.04 | 27.73±0.07 | 55.48±0.03 | 1.17±0.06 | 1.02 |
| | | 2-MG | 10.20±0.45 | - | - | 27.32±0.07 | 62.48±0.53 | - | |
| 5 | Partially Hydrogenated Soybean oils (USA) | TG | 10.38±0.04 | 0.26±0.01 | 5.10±0.02 | 47.10±0.04 | 36.32±0.028 | 0.84±0.01 | 0.74 |
| | | 2-MG | 7.74±0.176 | - | 5.46±0.28 | 43.59±0.40 | 43.23±0.516 | - | |

*PAEF= Palmitic acid (%) in 2-MG/Palmitic acid (%) in TG.

### C. Butter Fat

Four samples of butter fat collected from local market (3animal butter and 1 vegetable butter) were analyzed for the detection of lard. Data presented in Table XIII showed the fatty acid composition in TG, 2-MG and PAEF. It could be noticed that palmitic acid in 2-MG was higher than in TG position in animal butter (samples 1, 2 and 3), while it was lower in vegetable butter made from soybean, canola and palm oil (sample 4). Results indicated also PAEFs of animal butters were higher than vegetable butter. Abdel-kawy [28] and Al-Rashood [11] reported PAEFs of animal butter ranged from 1.03 to 1.23, while it was 0.64 in vegetable butter. The obtained results indicated that all samples of butter are free from lard according to their PAEFs Table XIII.

### D. Meat Products

Fatty acid compositions in TG, 2-MG and palmitic acid enrichment factors of 5 samples of meat products are listed in Table XIV. It is clear that the percent of palmitic acid is more at the position (2-MG) as compared to the TG of the first 4 samples (1, 2, 3 and 4, respectively). The PAEFs of these samples were found to be 1.62, 1.28, 1.08 and 1.39, respectively. The increase of C16:0 at 2-MG is only possible when lard or butter fat were present in the samples. Thus absence of characteristics short-chain fatty acids for butter fat clearly indicated that these samples contain lard. Sample (No 5) was free from lard or butter fat, indicated by lower level of C16:0 at 2-MG as compared to the TG and also low value of PAEF (0.51).

### E. Chocolates

Six samples of chocolates obtained from local market were analyzed for the detection of lard. Results in Table XV showed that Pamitic acid % in 2-MG was lower being, 9.46, 15.21, 17.45, 9.77, 15.82 and 17.11% than that in TG being 37.76, 39.57, 34.62, 26.66, 36.88, and 37.32 % for samples 1 , 2, 3, 4, 5 and 6, respectively. On the other hand, PAEF was being 0.25, 0.38, 0.50, 0.37, 0.43 and 0.46, respectively. As reported on the labels of chocolates, it could be noticed that, sample (1) made from palm oil, canola oil, linseed oil and milk fat. Zock et al. [29] reported that palmitic acid esterifies by approximately 29 % and 7% on TG and 2-MG of palm oil, respectively. However, canola oil contains 4.70-5.30% palmitic acid in TG and 1.3% in 2-MG as reported by [30], [1], [26]. Linseed oil contains 6% and 2% palmitic acid esterifies in TG and 2-MG, respectively [26]. Sample (2) was made from palm oil, cocoa butter and milk fat. It was reported that Cocoa butter contains C16:0 (24-26%), C18:0 (35-37%), C18:1 (33-36%) and C18:2 (2.3-3%) esterifies in TG as mentioned by [31], [32], [26]. Moreover, Pamitic acid esterifies at 2-MG by 2- 5 % as reported by [26]. Sample (3) made from roasted peanuts, palm fat, milk fat and cocoa butter

Open Science Index, Nutrition and Food Engineering Vol:8, No:11, 2014 publications.waset.org/10000133/pdf

as reported on its label. The fatty acids of peanut were previously analyzed by [26]. They reported that C16:0 in TG of peanut was 11% while it was 2% in 2-MG. On the other hand, sample (4) made from Cocoa butter, sunflower oil and palm oil. Nasyrah et al. [33] reported that sunflower oil contains 7.98 % and 1 % palmitic acid in TG and 2-MG, respectively. Samples (5 and 6) made from Cocoa butter, palm oil and milk fat. It could be noticed that, all samples of chocolates under investigation contains milk fat as reported on its labels. Milk fat contained short chain fatty acids (C8:0 – C10:0) either in TG or 2-MG as shown in Table XV. Mansson [34] and Al-Rashood et al. [11] reported that milk fat contains 30% palmitic acid in TG and 35% in 2-MG. Palmitic acid enrichment factors of all chocolate samples (ranged from 0.25 to 0.50) as shown in Table XV. Thus, it could be concluded that all samples under investigation free from lard as confirmed by low PAEF.

TABLE XIII
FATTY ACID COMPOSITION IN TRIACYLGLYCEROL (TG), 2-MONOACYL- GLYCEROL (2-MG) AND PALMITIC ACID ENRICHMENT FACTOR (PAEF) OF SOME ANIMAL AND VEGETABLE BUTTER COLLECTED FROM LOCAL MARKET

| No | Coded samples | | Fatty acids (%) | | | | | | | | *PAEF |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | C10:0 | C12:0 | C14:0 | C16:0 | C18:0 | C18:1 | C18:2 | C18:3 | |
| 1 | Animal butter (Cream from cow's milk fat) (Denmark) | TG | 2.31±0.09 | 2.87±0.31 | 10.37±0.24 | 34.24±0.61 | 14.22±0.22 | 33.78±0.12 | 2.21±0.14 | - | |
| | | 2-MG | 9.00±0.31 | 4.52±0.38 | 6.70±0.65 | 39.50±0.09 | 6.53±0.06 | 21.38±0.65 | 2.37±0.65 | - | 15 |
| 2 | Animal butter (Cream from cow's milk fat) (France) | TG | 2.39±0.247 | 2.89±0.14 | 10.89±0.41 | 37.30±0.22 | 12.78±0.17 | 31.73±0.37 | 2.02±0.02 | - | 1.18 |
| | | 2-MG | 6.88±0.735 | 4.30±0.16 | 19.06±0.12 | 44.36±0.07 | 5.20±0.19 | 18.22±0.64 | 1.98±0.14 | - | |
| 3 | Animal butter (Cream from cow's milk fat) (Irish) | TG | 2.48±0.141 | 2.74±0.13 | 9.90±0.28 | 30.05±0.04 | 15.75±0.02 | 37.18±0.53 | 1.90±0.09 | - | 1.53 |
| | | 2-MG | 1.99±0.014 | 5.22±0.25 | 18.25±0.20 | 46.08±0.09 | 4.44±0.31 | 15.19±0.28 | 8.83±0.46 | - | |
| 4 | Vegetable butter (Soybean, Palm and Canola oils) (USA) | TG | - | 0.59±0.01 | 0.52±0.01 | 12.48±0.20 | 5.71±0.01 | 27.61±0.04 | 47.26±0.21 | 5.83±0.07 | 0.35 |
| | | 2-MG | - | - | 2.10±0.12 | 4.39±0.38 | 5.86±0.01 | 28.21±0.12 | 55.36±0.19 | 4.08±0.20 | |

*PAEF= Palmitic acid (%) in 2-MG/Palmitic acid (%) in TG**.**

TABLE XIV
FATTY ACID COMPOSITION IN TRIACYLGLYCEROL (TG), 2-MONOACYL- GLYCEROL (2-MG) AND PALMITIC ACID ENRICHMENT FACTOR (PAEF) OF SOME MEAT PRODUCTS COLLECTED FROM LOCAL MARKET

| No | Coded samples | | Fatty acids (%) | | | | | | | *PAEF |
|---|---|---|---|---|---|---|---|---|---|---|
| | | | C14:0 | C16:0 | C16:1 | C18:0 | C18:1 | C18:2 | C18:3 | |
| 1 | Chicken Luncheon canned meat (Brazil) | TG | 0.42±0.07 | 12.58±0.03 | 4.10±0.02 | 7.93±0.07 | 45.17±0.08 | 28.83±0.07 | 0.97±0.07 | 1.62 |
| | | 2-MG | - | 20.43±0.12 | 3.74±0.05 | 6.06±0.19 | 49.01±0.19 | 20.76±0.17 | - | |
| 2 | Chicken Vienna canned sausage (Brazil) | TG | - | 14.98±0.31 | 4.17±0.15 | 7.30±0.04 | 44.88±0.29 | 27.52±0.12 | 1.15±0.01 | 1.28 |
| | | 2-MG | - | 19.19±0.02 | 4.10±0.03 | 6.08±0.03 | 49.98±0.05 | 20.65±0.07 | - | |
| 3 | Chicken Vienna canned sausage (Brazil) | TG | 0.42±0.02 | 20.30±0.19 | 4.36±0.05 | 7.71±0.06 | 43.95±0.15 | 22.31±0.01 | 0.95±0.07 | 1.08 |
| | | 2-MG | - | 21.95±0.26 | 4.14±0.06 | 5.88±0.21 | 46.42±0.02 | 21.61±0.07 | - | |
| 4 | Frozen Turkey meat loaf (USA) | TG | 3.30±0.04 | 21.40±0.24 | 1.94±0.21 | 7.61±0.13 | 35.20±0.19 | 27.35±0.01 | 3.20±0.19 | 1.39 |
| | | 2-MG | 1.82±0.02 | 29.74±0.07 | 0.83±0.01 | 11.28±0.16 | 37.98±0.07 | 18.35±0.26 | - | |
| 5 | Chicken Luncheon meat (Netherland) | TG | 0.57±0.19 | 19.97±0.71 | 3.90±0.36 | 6.54±0.33 | 45.56±0.46 | 20.99±0.42 | 2.47±0.04 | 0.51 |
| | | 2-MG | 0.91±0.03 | 10.08±0.25 | 3.96±0.13 | 6.60±0.06 | 51.86±0.31 | 24.89±0.20 | 1.70±0.01 | |

*PAEF= Palmitic acid (%) in 2-MG/Palmitic acid (%) in TG

TABLE XV
FATTY ACID COMPOSITION IN TRIACYLGLYCEROL (TG), 2-MONOACYL- GLYCEROL (2-MG) AND PALMITIC ACID ENRICHMENT FACTOR (PAEF) OF SOME CANDY PRODUCTS (CHOCOLATES) COLLECTED FROM LOCAL MARKET

| o | | Fatty acids (%) | | | | | | | | | | *PAEF |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | C8:0 | C10:0 | C12:0 | C14:0 | C16:0 | C16:1 | C18:0 | C18:1 | C18:2 | C18:3 | |
| | TG | - | 0.70±0.01 | 2.36±0.91 | 6.40±0.01 | 37.76±0.50 | 0.41±0.11 | 5.22±0.66 | 36.42±0.30 | 9.61±0.12 | 1.12±0.06 | 0.25 |
| | 2-MG | - | 0.91±0.02 | 1.70±0.01 | 4.41±0.21 | 9.46±0.07 | 0.31±0.01 | 3.58±0.23 | 52.67±0.65 | 23.82±0.51 | 3.14±0.04 | |
| | TG | - | - | 0.19±0.01 | 1.01±0.01 | 39.57±0.11 | 0.29±0.02 | 13.41±0.27 | 35.10±0.35 | 9.46±0.01 | 0.97±0.02 | 0.38 |
| | 2-MG | - | - | 0.51±0.11 | 2.40±0.21 | 15.21±0.12 | 1.14±0.01 | 14.65±0.23 | 53.71±0.45 | 12.38±0.17 | - | |
| 3 | TG | 1.90±0.0 | 1.00±0.10 | 2..80±0.08 | 3.20±0.12 | 34.62±0.12 | 3.00±0.12 | 10.97±0.12 | 39.00±0.22 | 3.51±0.02 | - | 0.50 |
| | 2-MG | 2.47±0.48 | 1.64±0.05 | 3.13±0.18 | 3.11±0.16 | 17.45±0.30 | 5.41±0.04 | 10.31±0.19 | 52.31±0.08 | 4.17±0.08 | - | |
| 4 | TG | - | - | 0.19±0.01 | 0.66±0.19 | 26.66±0.22 | 0.31±0.05 | 22.63±0.78 | 34.45±0.62 | 13.87±0.14 | 1.23±.0.05 | 0.37 |
| | 2-MG | - | - | 0.69±0.01 | 1.16±0.14 | 9.77±0.32 | 1.31±0.02 | 22.22±0.11 | 46.60±0.91 | 16.02±0.12 | 2.23±0.02 | |
| 5 | TG | - | 0.16±.0.01 | 0.36±.0.01 | 1.87±0.04 | 36.88±0.05 | 0.51±0.08 | 15.61±0.44 | 39.50±0.34 | 4.30±0.01 | 0.81±0.01 | 0.43 |
| | 2-MG | - | 0.28±0.01 | 1.31±0.14 | 2.29±0.25 | 15.82±0.48 | 2.16±0.15 | 14.99±0.24 | 52.76±0.78 | 8.93±0.34 | 1.46±0.03 | |
| 6 | TG | - | 0.17±0.01 | 1.05±0.02 | 1.58± 0.40 | 37.32±0.26 | 0.31±0.11 | 17.11±0.63 | 36.84±0.85 | 4.39±0.05 | 1.23±0.04 | 0.46 |
| | 2-MG | - | 0.26±0.03 | 1.28±0.11 | 2.13±0.14 | 17.11±0.05 | 1.18±0.41 | 20.18±0.47 | 46.60±0.90 | 9.12±0.11 | 2.14±0.02 | |

*PAEF= Palmitic acid (%) in 2-MG/Palmitic acid (%) in TG**.**

**1-** Moist tender coconut covered in thick milk chocolate made from Palm oil, Canola oil, Linseed oil and milk fat (Netherlands**).**
**2:** Milk chocolate with caramel and biscuit made from Palm oil, Cocoa butter, milk fat (Netherlands)**.**
**3:** Milk chocolate with soft nougat made from Palm oil, Cocoa butter, roasted peanut, milk fat (UAE).
**4:** Milk chocolate with a light whipped white center made from Sunflower oil, Palm oil and Cocoa butter (Russia)
**5:** Filled milk chocolate with a rich milky filling made from Palm oil, Cocoa butter, milk fat (Germany)**.**
**6:** Maltesers made from Palm oil, Cocoa butter, milk fat (Netherlands).

Open Science Index, Nutrition and Food Engineering Vol:8, No:11, 2014 publications.waset.org/10000133/pdf

From the aforementioned results it could be concluded that palmitic acid enrichment factor (PAEF) has been taken as a detection merit for crude lard, either singly or admixed with other fats and oils or in fatty foods. As mentioned previously, [24], [35]-[37] reported that the increase of palmitic acid enrichment factor up to 0.5 as well as a decrease of unsaturation ratio to 1.3 or less indicated the presence of 5 % pork fat or more.

## ACKNOWLEDGMENT

This research has been supported by project grant from the National Plan for Science and Technology, King Saud University. Thanks and appreciations are extended to all research team for their efforts and hard work.

## REFERENCES

[1] J.M.N. Marikkar, H.M. Ghazali, Y.B. Che Man, T.S.G. Peiris, and O.M. Lai, "Distinguishing lard from other animal fats in admixtures of some vegetable oils using liquid chromatographic data coupled with multivariate data analysis," Food Chem., vol. 9, pp.5-14, 2005.

[2] A. Rohman, and Y.B. Che Man, "Fourier transform infrared (FTIR) spectroscopy for analysis of extra virgin olive oil adulterated with palm oil," Food Res. International, vol. 43, pp.886-892, 2010.

[3] J. M. Regenstein, M. M. Chaudry, and C. E. Regenstein, "The kosher and halal food laws," Comprehensive Reviews in Food Science and Food Safety, vol. 2, pp. 111–127, 2003.

[4] F.H .Mattson, R.A. Volpenhein, and E.S. Lutton, "The distribution of fatty acids in the triglycerides of the Artiodactyla (even-toed animals)," J. Lipid Res., vol. 5, pp. 3636-365, 1964.

[5] R.S. Hal, L. Elaine, H.T. Raymond, S.D. Cavol, and V.M. George, "Lipid in raw and cooked beef," J. Food composition and Analysis, pp.26-37, 1987; International Union of pure and Applied Chem., Applied Chem. Division, Commission on Oils, Fats and Derivates, 6th Ed. part 1 (section I and II), 1979. Pergamon Press, New York.

[6] G.K. Chacko, and E.G. Perkins, "Anatomical variation in fatty acid composition and triglyceride distribution in animal depot fats," J. Am. Oil Chem. Soc., vol. 42, pp.1121-1124, 1964.

[7] B. Jayme, M.N. Reddy, and R.G. Alssa, "A simple method for the separation of triacylglycerols from fatty acids released in lipase assays," J. Lipid Res., vol.29, pp.1549-1552, 1988.

[8] IUPAC, "International Union of Pure Applied Chemistry. Commission on Oils, Fats and Derivatives, Standard Methods for the Analysis of Oils," 6th edn. Pergamon, New York, Method 2.210, 1979.

[9] A.H. Bayoumy, "Studies on the detection pork products in some foods," M.Sc. Thesis, Faculty of Agri., Moshtohor, Zagazig Univ., Egypt, 1982.

[10] Y.M. Youssef, M.B. Omer, A. Skulberg, and M. Rashwan, "Detection and evaluation of lard in certain locally proceeded and imported meat products," J. Food Chem., vol.30, pp.167-180, 1988.

[11] K.A. Al-Rashood, R.R.A. Abu-Shaaban, E.M. Abdel-Moety, and Abdul-Rauf, "Compositional and thermal characterization of genuine and randomized lard: A comparative study," AOCS, vol.73, no.3, pp.303-309, 1996.

[12] T. Dourtoglou, E. Stefanou, S. Lalas, V. Dourtoglou, and C. Poulos, "Quick regiospecific analysis of fatty acids in triacylglycerols with GC using 1, 3-specific lipase in butanol, Analyst, vol.126, pp.1032–1036, 2001.

[13] W. W. Christie, "The positional distributions of fatty acids in triglycerides. In R. J. Hamilton & J. B. Rossell (Eds.), "Analysis of oils and fats, pp. 313–339, 1986, Essex: Elsevier Applied Science.

[14] W. W. Christie, "The positional distributions of fatty acids in triglycerides. In R. J. Hamilton & J. B. Rossell (Eds.), "Analysis of oils and fats, pp. 313–339, 1986, Essex: Elsevier Applied Science.

[15] T. Saeed, S. G .Ali, H. A. A. Rahman, and W. N. Sawaya, "Detection of pork and lard as adulterants in processed meat: Liquid chromatographic analysis of derivatized triglycerides," J. of the Association of Official Analytical Chemists, vol.72, pp.921–925, 1989.

[16] M. A. Soliman, and N. A. Younes, "Adulterated butterfat: Fatty acid composition of triglycerides and 2-monoglycerides," J. of the American Oil Chemists Society, vol. 63, pp.248–250, 1986.

[17] E. Dister, and F.J. Baur, "The determination of mono, di- and triglycerides concentrates by column chromatography," J. Assoc. Agric. Chem., vol. 48, no.2, pp.444-448, 1965.

[18] W.R. Morrison, and L.M. Smith, "Preparation of fatty acid methyl esters and dimethylacetals from lipids with boron fluoride-methanol," J. Lipid Res., vol. 53, pp.600-608, 1964.

[19] IUPAC, "International Union of Pure Applied chemistry. Determination of fatty acid in the 2-position in the triglycerides of oils and fats," In Standard Methods for the Analysis of oils, fats and derivatives, edited by C. Paquoec and A. Hautfenne. Blockwell Scientific publishers Ltd. P.111. 1987.

[20] F. E. Luddy, R.A. Barford, S.F. Herb, and P. Magidman, "A rapid and quantitative procedure for the preparation of methyl esters of butter oil and other fats," J. Am. Oil Chem. Soc., vol. 45, pp. 549-553, 1968.

[21] W.W. Christie, and J.H. Moore, "Structures of triglycerides isolated from various sheep tissues," J. Sci. Food. Agric., vol. 22, pp.120-124, 1971.

[22] A.M.F. Abou-Hadeed, and A.R. Kotb, "Detection of small amounts of lard in other animal fats," Qatar Univ. Sci. J., vol.13, no. 1, pp. 30-39, 1993

[23] A. Uzzan, "Oleaginous Fruits and their oils, In: Oils and Fats Manual, A Comprehensive Treatise," Vol.1.Karleskind, A. (Ed), pp. 225-233. 1996, Lavoisier Publishing, ISBN: 1-898298-08-4.Paris, France.

[24] A.A. Abou Arab, "Identification of the sort of fats and oils in different foods," M.Sc. Thesis, faculty of agric. Ain shams Univ., Cairo, Egypt, 1980.

[25] J. Folch, M. Lees, and G.H. Sloane-Stanley, "A simple method for the isolation and purification of total lipids from animal tissues," Biol. Chemist's, vol. 226, pp.497. 1957.

[26] W.W. Christie, B. Nikolova-Damyanova, P. Laakso, and B. Herslof, "Stearospecific analysis of triacyl-sn-glycerols via resolution of diasteromeric diacyleglycerol derivatives by high-performance liquid chromatography on silica," J. Am. Oil Chemist's Soc., vol.68, pp.695-701, 1991.

[27] P. Angers, and J. Arul, "A simple method for regiospecific analysis of triacylglycerols by gas chromatography," J. AOCS, vol. 76, no.4, pp.481-484, 1999.

[28] M.I. Abdel-kawy, "Analysis of some edible fats and fatty foods for the detection of pig fat," Ph.D. Thesis (Pharm. Sci., analytical Chemistry), faculty of Pharmacy, Cairo University, 1987.

[29] P.L. Zock, H.M. Jeanne, J. Nanneke, and B.K. Martijn, "Positional distribution of fatty acids in dietary triglycerides: effects on fasting blood lipoprotein concentrations in humans," Am. J. Clin. Nut., vol. 61, pp.48-55, 1995.

[30] L.Z. Cheong, H. Zhang, L. Nersting, K. Jensen, J.A.J. Haagensen, and X. Xu, "Physical and sensory characteristics of pork sausages from enzymatically modified blends of lard and rapeseed oil during storage," Meat Sci., vol. 85, pp. 691–699, 2010.

[31] I. Foubert, P.A. Vanrolleghem, O. Thas, and K. Dewettinck, "Influence of Chemical Composition on the Isothermal Cocoa Butter Crystallization," J. Food Sci., vol.69, no. 9, pp.478-487, 2004.

[32] A. Neri, M.S. Simonetti, L. Cossignani, and P. Damiani, "Identification of cocoa butter equivalents added to cocoa butter I. An approach by fatty acid composition of the triacylglycerol sub-fractions separated by Ag+-HPLC," Zeitschrift Fur Lebensmittelunterschung und Forsschung A., vol.206, no. 6, pp.387-392, 1998.

[33] A.R. Nasyrah, J.M.N. Marikkar, and M.H. Dzulkifly, "Discrimination of plant and animal derived MAG and DAG by principal component analysis of fatty acid composition and thermal profile data," Inter. Food Res. J., vol. 19, no.4, pp.1497-1501, 2012.

[34] H.L. Mansson, "Fatty acid in bovine milk fat," Food Nutr. Res., vol. 52, pp.10-34, 2008.

[35] L.E. Abdel-Fattah, "Analysis study of some food and pharmacutica lipids products," Ph.D. thesis, Faculty of Pharmacy, Cairo Univ., 1974.

[36] L. El-Sayed, A.K.S. Ahmed, and M.N. Amer, "The detection of lard in hydrogenated fats," La Riv. Ital. Della Sostanze Grasse, vol.62, pp.553, 1985.

[37] A. Thielemans, H .De-Brabander, and D.L Massar, "Class definition and mixture class definition by means of construction of convex hull boundaries application to analysis for animal fat adulteration," J. Assoc. Off. Anal. Chem., vol.72, pp.41-47, 1989.

Open Science Index, Nutrition and Food Engineering Vol:8, No:11, 2014 publications.waset.org/10000133/pdf