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Investigation of Trans Fatty Acids and Saturated Fatty Acids Presence in Selected Bakery and Fast Food Products

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Abstract: Modern trends in food habits are moving towards fast food outlets due to the busy lifestyle of people all over the world. Due to consumption of foods with excessive amount of Trans Fatty Acids (TFA), may leads to heart and other chronicle diseases. Therefore, with a view to study presence of TFA and changes occurrence on fatty acid profile during baking and cooking, thirteen bakery and fast food products were selected for this study. These products were purchased from premier food outlets in Sri Lankan market and subjected to analyze TFA and SFA by adapting respective protocols relevant to derivatization by ester formation, gas chromatography-mass spectrometry for individual separation and identification following total fat isolation by microwave-assisted soxhlet extraction.
Results of the study revealed that total fat percentages of the bakery products and fast foods were ranging from 3.705 ± 0.224 to 26.732 ± 0.662 and from 7.805 ± 0.230 to 14.546 ± 0.477 respectively. Based on the fatty acid profile analysis and the availability of SFAs of all the products were ranging from 20.543 ± 0.334 to 43.109 ± 0.411and TFAs from 20.758 ± 0.239 to 39.975 ± 0.377. However, two bakery products namely fish bun and fish patty did not contain TFAs. According to this study, availability of TFA and SFA in most of processed foods were (E)-octadec-9-enoic acid (elaidic acid) and hexadecanoic acid (palmitic acid) respectively.

Keywords: Trans fatty acids, Saturated fatty acids, Fast foods, Bakery products, Fatty acid profile

1. INTRODUCTION

Trans fatty acids (TFAs) means the sum of all unsaturated fatty acids which contains at least one non-conjugated and trans double bond**.** The double bond in the transconfiguration gives the molecule a straighter shape. The straighter shape of the molecules allows them to pack better in the solid-state. This leads to a transformation of oils into semisolid fats [1,2,3].

World Health Organization (WHO) recommends that the total TFA intake to be limited to less than 1% of total energy intake, or less than 2.2g per day with a 2000 calorie diet. Trans fats cause to increase the levels of LDL-cholesterol which is a well-accepted biomarker for

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cardiovascular disease risk and decrease levels of HDL-cholesterol causes to carry away cholesterol from arteries and transport it to the liver that secretes it into the bile [2]. Replacing trans-fat with unsaturated fatty acids (UFA) decreases the risk of heart disease by ameliorating the negative effects of TFA on blood lipids and also, there are indications that trans-fat may increase the inflammation and endothelial dysfunction [3]. Further, trans fats contribute to heart disease by raising the levels of LDL (bad cholesterol) and lowering the levels of HDL (good cholesterol) by damaging the cells in the linings of blood vessels, which lead to inflammation and blockage and ultimately to heart attacks [3].

The TFAs in common vegetable oils such as corn, peanut, olive, and soybean oil are mainly compounding of 18 carbon atoms with 1-3 double bonds [4,5]. Elaidic acid (9-trans-C18:1) is a typical industrial TFA, produced by partial hydrogenation of vegetable oil. Vaccenic acid (11-trans-C18:1) is the predominant TFA in milk and meat from ruminant animals, although small amounts are also found in industrially hydrogenated fats [5].Variation of TFA content is depending on food to food and even within the same food category. But health implications from TFA depend on consumption patterns and other factors. Foods that contained high percentage of TFA are frying fats/ partially hydrogenated vegetable oils(PHVOs), shortenings & margarine, bakery products (cookies, crackers, cakes, biscuits, wafers &, etc.), microwave popcorn, deep-friedfoods, fast foods, frozen foods and traditional deep-fried foods [6,7,8]. In the case of Sri Lankan context, where fast foods are generally cheap, accessible and delicious. According to the study conducted by the Medical Research Institute, Sri Lanka in 2012 has brough into the light that around 50% of the people who regularly take meal from out side rather than home-prepared and these foods may contain high calory value constituents including fat and oils which may be enclosed with TFA and SFA too. Therefore, endeavor of this study was to quantify the presence of trans fatty acid and fully saturated fatty acids in selected food products, produced by the major food outlets in Sri Lanka.

2. MATERIALS AND METHODS

a. Sampling Plan

Thirteen number of processed food items including both bakery and fast-moving food products were collected randomly according to higher consumer preference as well as products contained a high percentage of fat (probably 5% >) from the leading bakery and fast food outlets in Colombo District, Sri Lanka and these food products were kept under refrigerator conditions (4-6°C). Oil extraction from collected materials was carried out for 3-4 hours by using microwave assistedsoxhlet extraction [9]and collected oil samples were stored in dark amber color bottles without headspace at -18°C for subsequent use of this study.

b. Determination of Total Fat Content of Collected Food Products

Total fat content of collected food products was determined according to the methodology of [10].Therein,20-30g of the sample was weighed using the analytical balance (GRAM, FR-500) and placed into Whatman thimble. Fat extraction was carried out using the soxhlet apparatus for 3-4hr into a flask containing 150ml of n-hexane (GC-MS suprasolv) (as a replacement for pet-ether). After the extraction, solvent was evaporated using the rotary evaporator and the fat residue was weighted to get the total fat content of the sample. All samples were replicated thrice in order to get the mean oil.

c. Determination of Fatty Acid Profile of Extracted Oil from Different Type of Food Products

In determining fatty acid profile of extracted oils, fatty acid methyl esters (FAMEs) were prepared according to the method of Industrial Technology Institute (ITI), Sri Lanka compatible to [11] with slight modification to increase the accuracy of derivatization. Therein, approximately l g of oil was weighed into a clean& dry beaker. Three milliliter (3ml) of toluene and 1.5ml of Sodium Methoxide solution were added into it and mixed well. Thereafter, 4.5ml of methanol was added into the mixture and again mixed well. Then the beaker with the mixture was kept on a hot plate at 50°Cfor about 15 minutes. After that beaker withthe mixture was allowed to cool down to room temperature and added 10ml of distilled water and shake well. Thereafter, 9ml of hexane was added into the mixture and mixed well. Mixture was allowed to stand for about 10 minutes to separate water & hexane layers. After separation, upper hexane layer was transferred from a dropper to test tube which contained sodium sulfate anhydrous to remove any traces of moisture. Finally FAME was transferred to a capped and labeled glass vial using GC filters and syringes for GC-MS analysis.

Fatty acid profiles were determined in the FAMEs by using a Gas Chromatograph (model 7890 A, agilent technologies) equipped with Mass Spectrometer (model 5975 C inert XL El/CI MSD) along with triple-axis detector. The carrier gas was helium at a flow rate of 1ml/min (split ratio 30:1). FAMEs were separated by using a BPX-70 fused silica capillary column (30 M, 0.25mm i.d., 0.2 µm film thickness, Melbourne, Australia). The injected volume of the sample was 1 µL. The initial column temperature was l00°C and thereafter it was programmed to increase 10°C/min until the temperature reach to 160°C, thereafter 3°C/min to 220°C and finally 10°C/min until 260°C. The total run time of the sample was about 45 minutes.

The different fatty acids in FAMEs were identified by the comparison of the retention times of the peaks in the samples versus standard compounds. Individual pure methylated standards from Sigma Aldrich were used in order to identify the trans fatty acids, saturated fatty acids and remaining other mono and polyunsaturated fatty acids of the samples. The concentrations of the identified fatty acids of the samples were qualitatively determined with respect to the peak areas of the chromatograms. All FAMEs were replicated thrice.

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d. Statistical Analysis

All collected data were statistically analyzed by using Minitab statistical package (version 17) with respect one way ANOVA followed by Tukey b posteriori test to determine the significance difference among the products. A significance level of P<0.05 was used for all evaluations.

3. RESULTS AND DISCUSSION

e. Determination of Total Fat Content of Collected Food Products

Total fat content of collected food products was determined as a percentage as well as amount per serving size for short (120g<) and major meals (120g>) and the results are illustrated in table 01 and table 02.

Table 01 -Total Fat Content of Short Meals

Sample Code	Name of product	Oil content per 100g (X±SD)	Oil content per serving (X±SD)	Serving size
G1	Sausage Pastry	25.499 ± 0.991^{a}	15.299 ± 0.595	60g
G2	Egg with Seeni Sambol Pastry	26.732 ± 0.662^{a}	16.304 ± 0.378	62g
G3	Fish Roll	8.584 ± 0.528^{b}	4.304 ± 0.219	50g
G4	Egg Roll	11.122 ± 1.260^{c}	12.588 ± 1.444	112g
G5	Fish Bun	3.705 ± 0.224^{d}	2.805 ± 0.176	75g
G6	Fish Patty	9.338 ± 0.767^{b}	4.198 ± 0.263	45g

Different letters indicate significantly different values (p<0.05)

Table 02 - Total fat Content of Major Meals

Sample Code	Name of product	Oil content per 100g (X±SD)	Oil content per serving (X±SD)	Serving size
G7	Devilled C/P – Outlet 1	9.159 ± 1.424^{a}	23.04 ± 0.349	250g (Small)
G8	Devilled C/P – Outlet 2	7.805 ± 0.230^{a}	18.704 ± 0.530	240g (Small)
G9	French Fries – Outlet A	12.804 ± 0.500^{b}	16.440 ± 0.971	130g (large)
G10	Fried Chicken – Outlet A	$13.953 \pm 0.463^{b,c}$	20.942 ± 1.322	150g
G11	French Fries –	12.467 ± 0.356^{b}	17.595 ± 0.899	125g (large)

	Outlet B			
G12	Fried Chicken – Outlet C	12.485 ± 0.946^{b}	18.120 ± 1.830	140g
G13	French Fries – Outlet C	14.546 ± 0.477^{c}	19.137 ± 0.504	130g (large)

Different letters indicate significantly different values (p<0.05)

The data given in the table 01 and 02 were statistically analyzed and found that there was a significant difference between food type and oil content of the all samples because the calculated p-value (0.0000) was less than (0.05) at 95% confidence level. According to the data given in table 01, while the highest oil contents were given in egg with seeni sambol pastry (26.732% ± 0.662) and sausage pastry (25.499% ± 0.991) the lowest fat contents were in fish bun and fish roll. However, egg roll and fish patty contained a moderate amount of fat. The reason for high percentage of fat in pastries was due to application of a thick layer of shortening over the dough tape at the beginning and thereafter it was folding into 4-6 layers in order to get a crispy-baked product. Therefore, consumption of more pastries per day should be avoided for a better healthy life.Moreover, consumption of selected major meals should also be limited because of the higher serving size as well as frequency of intake of these foods may cause for health maladies. Consumption of high-fat percentages, around 9-15% daily may cause for coronary heart diseases [12]. Further, fat is not the primary energy source of the body and consumption of it excessively along with the lack of exercises may cause for fat deposition in the blood capillaries of the human body that harms human health.French fries and fried chicken are deep-fried food products of fast food outlets and these food products were categorized as major food of this study. Therefore, their serving size is relatively high and usually they carry around 12-15% of fat (table 02). The data given in Table 2 also illustrate that the fried chicken purchased from two different outlets contained almost same percentage of fat. For the preparation of submarines, burgers and twister meals which are popular in all fast food outlets also contain considerable amount of fat because major ingredient of these product is fried chicken. French fries are served as both major and side dishes of a major meal in fast food outlets in the world as a deep fried food product [13]. On the other hand, most of the fast food outlets do the oil replacement in the deep fryer intermittently without following the good frying practices [14]. Hence, there is a great possibility to increases the ‘bad’ oil availability in the final product which people tend to consume.

According to the previous studies, oil contents of processed foods are varied because, rate of fat absorption into the foods are depending on the frying time (longer the frying time, higher the fat absorption), the total surface area of the food (greater the surface area, absorb more fat), frying temperature, nature of the food and nature of the oil used. However, generally food absorb around 20% of oil at the processing [15,16]. The fat content among the bakery products usually varied in the range of 25-40% due to the inclusion of different kind of margarine and fat spreads [17].The findings of this study were also revealed that the total fat content of selected bakery products was varied from 3.7% (fish bun) to 26.7% (egg pastry).

Hence, results obtained from this study were also in compliance with the result of [18]. According to their research, fat content of potato French fries and pastries were in the range of 11-20% and 13-30% respectively. Moreover, [19] reported that fat content of pizza and burger (with fried chicken) was 10-35% and 20-35% respectively.

f. Determination of Development of Trans Fatty Acids in Processed Foods

Trans Fatty Acid (TFA) and Saturated Fatty Acid (SFA) contents of selected food products were analyzed according to GC-MS and results are given in table 03.

Table 3 - Percentages of TFA, SFA and Other Unsaturated Fatty Acids of Selected Food Products

Sample Code	Item	TFA (%)	SFA (%)	Other Fat (%)
G1	Sausage Pastry	32.580 ± 0.344^{b}	43.109 ± 0.411^{a}	24.311 ± 0.369
G2	Egg with Seeni Sambol Pastry	39.278 ± 0.295^{a}	35.894 ± 0.380^{b}	24.828 ± 0.298
G3	Fish Roll	36.342 ± 0.233^{a}	37.800 ± 0.410^{b}	25.858 ± 0.211
G4	Egg Roll	38.323 ± 0.218^{a}	35.920 ± 0.531^{b}	25.757 ± 0.433
G5	Fish Bun	N.D.	38.057 ± 0.723 ^{b}	61.943 ± 0.820
G6	Fish Patty	N.D.	23.399 ± 0.457^{c}	76.601 ± 0.460
G7	Devilled Chicken Pizza – Outlet 1	35.430 ± 0.230^{a,b}	32.774 ± 0.288^{b}	31.796 ± 0.322
G8	Devilled Chicken Pizza – Outlet 2	39.975 ± 0.377^{a}	35.762 ± 0.428^{b}	24.263 ± 0.455
G9	French Fries – Outlet **A**	37.287 ± 0.566^{a}	33.391 ± 0.573^{b}	29.322 ± 0.644
G10	Fried Chicken – Outlet **A**	20.758 ± 0.239^{c}	20.543 ± 0.334^{c}	58.699 ± 0.390
G11	French Fries – Outlet **B**	39.314 ± 0.455^{a}	36.853 ± 0.522^{b}	23.833 ± 0.390

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G12	Fried Chicken – Outlet **C**	36.898 ± 0.398^{a}	33.166 ± 0.310 ^{b}	29.936 ± 0459
G13	French Fries – Outlet **C**	31.488 ± 0.655^{b}	40.922 ± 0.599^{a,b}	27.590 ± 0.622

Other Fat group contains remaining MUFA: monounsaturated fatty acid; PUFA: polyunsaturated fatty acid; N.D: not detected)

The data given in the table 03 indicate that TFA contents of, Egg with seeni sambol pastry, Egg roll, Fish roll, French fries (outlet A & B), Fried chicken (outlet C) and Devilled chicken pizza (outlet 1 & 2) were 39.278 ± 0.295, 38.323 ± 0.218, 36.342 ± 0.233, 37.287 ± 0.566, 39.314 ± 0.455, 36.898 ± 0.398, 35.430±0.230 and 39.975 ± 0.377% respectively and these values were not significantly difference toeach other ($p>0.05$) too. While lowest TFA level of 20.758 ± 0.239 % was reported by fried chicken (outlet A), fish bun and fish patty devoid of it. Reason for not developing TFA of these two products may be due to the baking process which usually takes place in a baking oven at 190-220°C and also these two products having relatively low amount of water (low amount of water causes to rapid formation of the crust). Further, the shortenings used for these two products were mixed with the other ingredients especially flour which can act as a protective shield for fat during baking, of which (the shield) prevents the fat directly exposing to the heat energy. And also formed crust can act as a heat barrier or lump that prevents rapid transmission of heat towards the center of the products. Hence, there is very low or no possibility to form TFA in fish bun and fish patty during baking. Almost same sentiment has been expressed by [20] in developing lesser amount of TFA. However, bakery products also vulnerable to the development of TFA, especially pastries as result of inclusion of margarine and fat spread. Margarine is an industrially hydrogenated food products and therefore the product itself may contains a considerable amount of TFAs specially elaidic acid [21].

However, the other products (moisture content of these products generally exceed 70%) contained with a higher percentage of TFS are subjected to deep frying or cooking by immersing them in hot oil (usually at 180-190°C) except pastries and devilled chicken pizza. During deep frying, the oil is directly exposed to the heat energy of the fryer while liberating considerable amount of water as steam. Therefore, there is a great possibility to form TFAs in the frying oil itself in the fryer and this oil may penetrate into the products being deep fried. However, the reason for having low TFA level in the fried chicken of outlet A was frequently replacing the oil in the fryer comparatively fried chicken of outlet B. Hence, good frying practices particularly replacement of oil regularly from the fryer is very important in order to avoid forming of excessive amount of TFA. In the case of low TFA in French fries outlet C, is due to using of a modern state of air fryer which frying time and temperature can be controllable automatically for frying purpose. Hence frying is taking place under control condition, thus formation of TFA is relatively low. The same kind of findings have been reported by [22] too. Devilled chicken pizza also contained considerable amount of TFA because Devilled chicken pizza contained ample amount of deep fried chicken pieces and deep fried onion pieces. Hence, these pieces contribute to elevate TFS in it remarkably.

As far as saturated fatty acid contents of these products are concerned, all most all of these products contained a sizeable amount of SFA. While highest amount of SFA is in sausage pastry, moderate and relatively low levels given by egg with seeni sambol pastry, fish bun, fish roll, egg roll, devilled chicken pizza (outlet 01 and outlet 02), French fries (Outlet A, B, and C) & fried chicken (outlet C) and fried chicken (outlet A) & fish patty respectively. However, the SFA content in sausage pastry is significantly difference to the other products ($P<0.05$) because it contained a medium size chicken sausage, usually chicken sausages contained around 35-45% of saturated fatty acids [23]. The SFA contents between the two clusters (moderate and low) are also significantly difference to each other ($P<0.05$). Reason for this phenomenon is using palm oil or palm olein as the frying medium which contains around 40-50% of fully saturated fatty acids [24].

g. Validation of the Research Findings by GC-MS Chromatograms

To further validate the research findings of this study, GC-MS chromatograms relevant to the selected bakery and fast foods are given in fig1-13. According to [25], area under the curve of GC-MS chromatograms can be used to identify the presence of major fatty acids in the fatty acid profile too, becausethe peak areas occurred in the GC-MS Chromatograms are directly proportional to the concentrations of fatty acids.According to GC-MS chromatograms, all selected food products except fish bun and fish patty contained two major type of fatty acids which are Elaidic acid (18:1n9t Octadecanoic acid) and palmitic acid (Hexadecanoic acid). Elaidic acid is a Trans fatty acid, it can be formed during cooking or deep frying process. Further, considerable amount of TFA are presented in partially hydrogenated vegetable oils (PHVOs) which usually used to make some of bakery products. Palmitic acid is the major SFA in the plant based oils particularly palm oil, which is the most popular oil used in commercial scale deep fryers.

However, according to figure 13 relevant to French fries outlet C that contained a high amount of Lauric acid which is the major SFA found in coconut oil and palm kernel oil. Therefore this outlet presumably used palm kernel oil rather than coconut oil because it is expensive and some instances consumers doesn’t like the flavor of coconut oil. Although TFA was not detected in the fish bun and fish patty (figure 5 and figure 6), these two products contained an abundant amount of oleic acid (18:1n9c Octadecanoic acid). However it can be transformed into Elaidic acid thorough elaidinization (chemical reaction which alters the orientation of double-bonds from cis to trans, [26]. Researchers observed that naturally all unsaturated FAs in the plant oils are in cis-form, therefore no TFA naturally present in plant-based cooking oils. But most of the time high temperatures, repeated usage of the same oil and partial hydrogenation isomerize the naturally occurred cis form into trans double bond form [27].

Hence, there is a great possibility to develop an abundant amount of TFA during food processing and this development is depending on two factors namely “food factor” and “frying factor”. Food factor is depending on nature of food, moisture content of food, ingredients used in the food, presence of bread crumb on the raw food and area of the food exposed to heat. In the case of frying factor

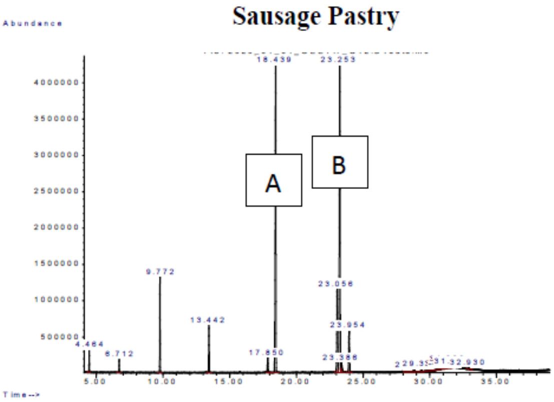

Figure 1: Fatty acid profile of sample G1
(18.439(A) = P.A (SFA), 23.253(B) = E.A (TFA))
(Note: P.A – Palmitic acid, E.A – Elaidic acid)

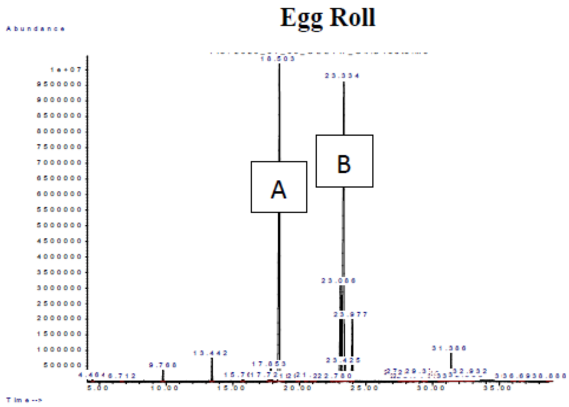

Figure 4: Fatty acid profile of sample G4
(18.503(A) = P.A (SFA), 23.334(B) = E.A (TFA))

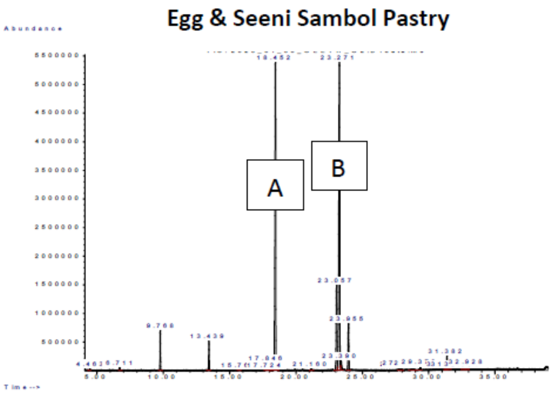

Figure 2: Fatty acid profile of sample G2
(18.452(A) = P.A (SFA), 23.271(B) = E.A (TFA))

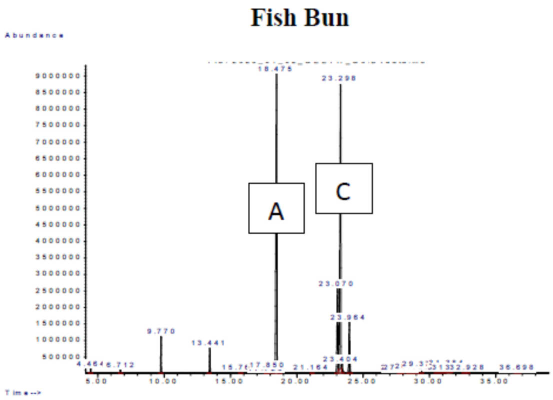

Figure 5: Fatty acid profile of sample G5
(18.475(A) = P.A (SFA), 23.298(C) = O.A (cis))
(Note: O.A – Oleic acid; the cis form of elaidic acid)

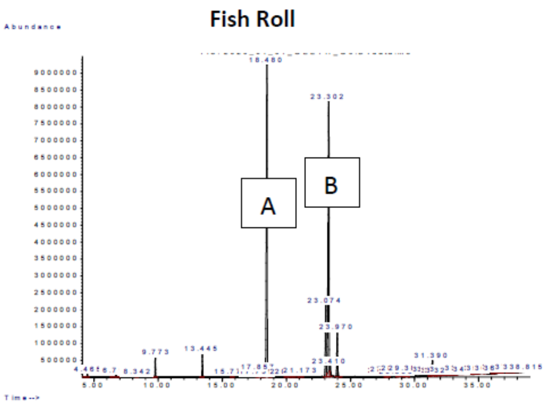

Figure 3: Fatty acid profile of sample G3
(18.480(A) = P.A (SFA), 23.302(B) = E.A (TFA))

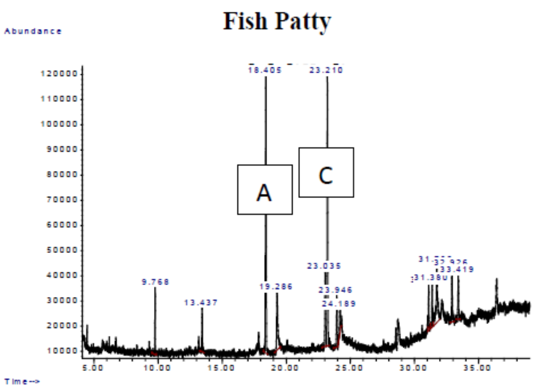

Figure 6: Fatty acid profile of sample G6
(18.405(A) = P.A (SFA), 23.210(C) = O.A (cis))

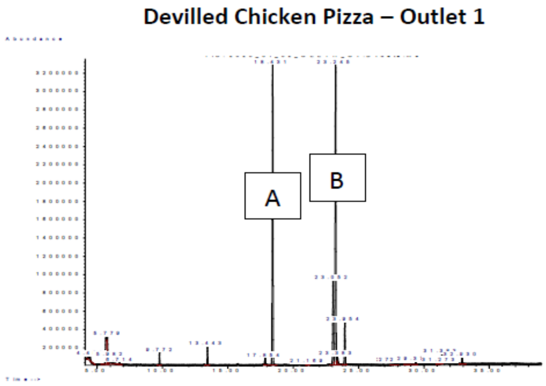

Figure 7: Fatty acid profile of sample G7
(18.431(A) = P.A (SFA), 23.245(B) = E.A (TFA))

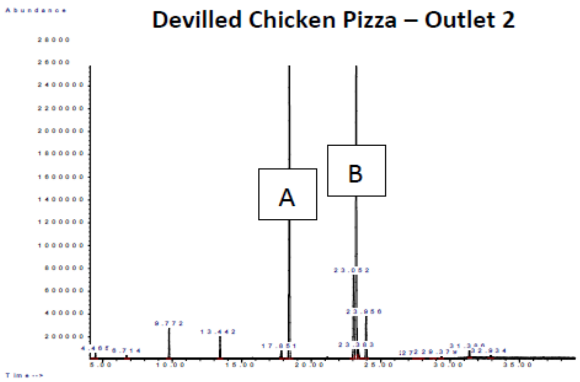

Figure 8: Fatty acid profile of sample G8
(18.428(A) = P.A (SFA), 23.241(B) = E.A (TFA))

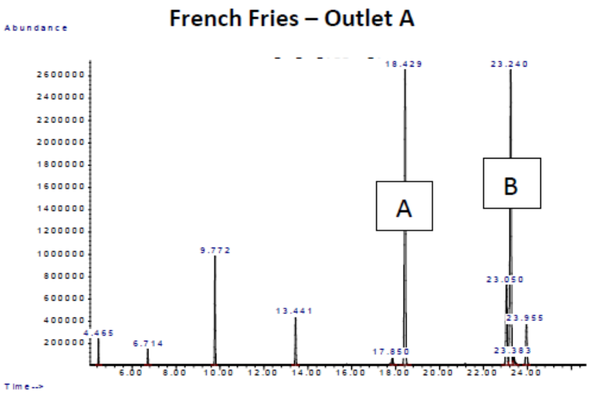

Figure 9: Fatty acid profile of sample G9
(18.429(A) = P.A (SFA), 23.240(B) = E.A (TFA))

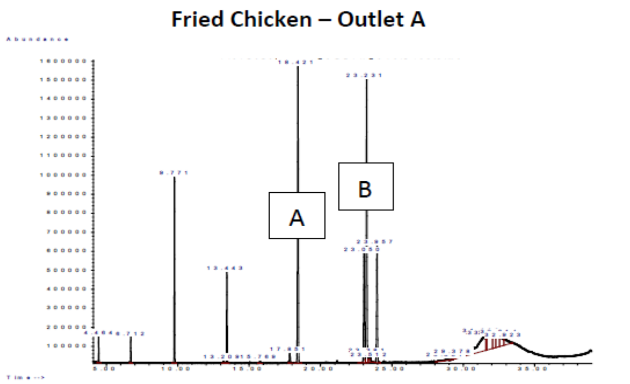

Figure 10: Fatty acid profile of sample G10
(18.421(A) = P.A (SFA), 23.231(B) = E.A (TFA))

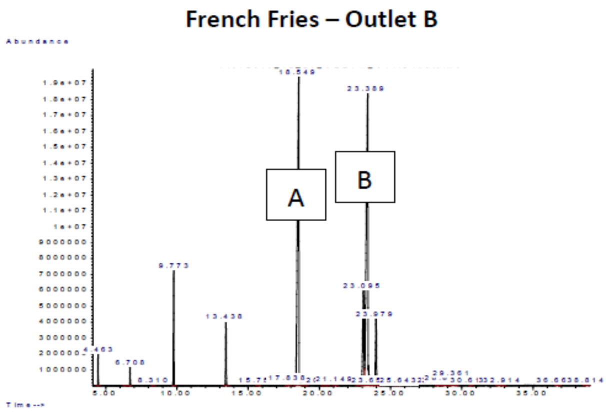

Figure 11: Fatty acid profile of sample G11
(18.549(A) = P.A (SFA), 23.389(B) = E.A (TFA))

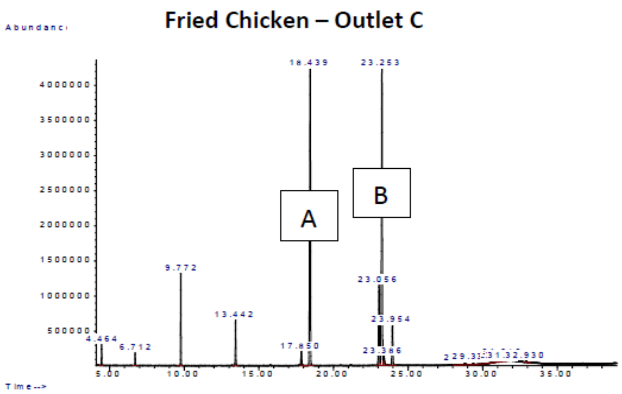

Figure 12: Fatty acid profile of sample G12
(18.439(A) = P.A (SFA), 23.253(B) = E.A (TFA))

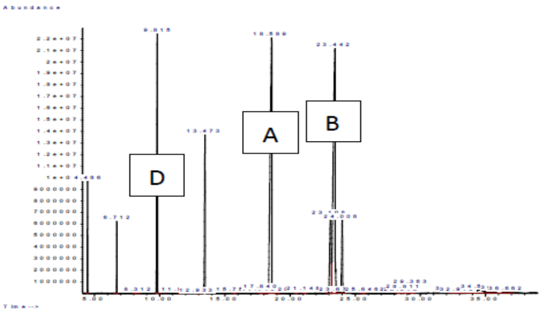

Figure 13: Fatty acid profile of sample G12
(9.815(D) = L.A (SFA), 18.589(A) = P.A (SFA),
23.442(B) = E.A (TFA))
(Note: L.A – Lauric acid, SFA with a 12-carbon atom chain)

which is depending on type of oil used in food processing, oil used for number of frying cycles, frequency of oil replacement, impurities presence in the frying oil, physicochemical properties of oil, (particularly Free Fatty Acid level, degree of saturation, moisture content, etc), oil contact surface of the fryer, good frying practices and performance of the fryer.

3. CONCLUSIONS

This study confirmed that TFAs are available in considerable amount of Sri Lankan bakery and deep fried food products. Highest amount of TFAs were found in all the bakery products except fish bun and fish patty, which did not contained TFA at all. Relatively, moderate and low amount of TFA were observed in sausage pastry & French fries (outlet C) and fried chicken (outlet C) respectively and all other products namely egg with seeni sambol pastry, fish roll, egg roll, devil chicken pizza (outlet1 and 2) and French fries (outlet A, B and C) contained comparatively high percentage of TFA. In the case of saturated fatty acids, highest amount of SFA was recorded by sausage pastry and comparatively moderate and low amount of SFA were shown by egg with seeni sambol pastry, fish roll, egg roll, fish bun, devil chicken pizza (outlet1 and 2) & French fries (outlet A, B and C) and fish fatty & fried chicken (outlet A) respectively.

Competing Interests

We declare that we have no competing interest.

Abbreviations

TFA – Trans Fatty Acid
SFA – Saturated Fatty Acid
PHVO – Partially Hydrogenated Vegetable Oil
GC – Gas Chromatography
MS – Mass Spectrometry

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