Trans Fatty Acids (TFAs) in Selected Processed Foods in Retail Markets of Morogoro

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Abstract

Globally, increased Trans Fatty Acids (TFAs) intake is estimated to be responsible for more than 500,000 deaths annually. There are consistent evidences of adverse health effects of industrial TFAs on blood lipoprotein profiles, coronary heart disease, cancer, diabetes, promotion of inflammation and endothelial dysfunction. In Tanzania, there is a sharp increase in Diet-Related Non Communicable Diseases (DR-NCDs). Reduction of TFAs in foods is considered as the most appropriate measure for tackling DR-NCDs in low and middle income countries. In May 2018, WHO announced strategic initiatives to make the world trans fatty acid free by 2023. The current study was conducted to determine the TFAs levels in "Chapatti", wheat buns, rice buns, potato chips and partially hydrogenated oils (PHOs) from retail shops in Morogoro region. A total of 60 samples were collected from two districts and analysed for total TFAs by Gas Chromatography Mass Spectrometry (GC-MS). Data was analysed using SPSS version 20.0. Means were separated by Tukey's honest Test at P≤0.05. Results indicated that all samples contained TFAs levels that were above the maximum recommended level by WHO of less than or equal to 2g/100g of fat, with the exception of "Chapatti". The highest amount of TFAs was observed in PHOs (5.69±0.042 g/100g fat). The mean TFAs concentration differed significantly among food types at P≤0.05). There was no significant effect of locations from where samples were collected on mean TFAs concentration at the same level. Other snacks in this study, when consumed on regular basis could add up more TFAs in our bodies thereby increasing the risks for DR-NCDs, which have become more prominent in the country. Tanzania should also join efforts to eliminate industrially produced TFAs by implementing legislative /regulatory actions to prohibit and/or limit their use in foods.

Keywords: *Trans* fatty acids, Partially hydrogenated oils, Cardiovascular disease, Morogoro

Introduction

TFAs are a type of unsaturated fatty acids classified as either naturally occurring or industrially produced (Kamel, *et al.,* 2018). Naturally occurring TFAs or ruminant *trans* fatty acids (rTFAs) are found in small proportions (3-6%) in food products such as meat and milk products from animals (Mouratidou *et al.,* 2014). Industrially produced *trans* fats (iTFAs) are formed by the process of partial hydrogenation in the industrial production of PHOs (Afaneh *et al.,* 2017), during the process oil is hardened, which improves its commercial appeal by enhancing its sensory and texture profiles, also increasing its shelf life and tolerance to repeated heating (Mouratidou *et al.,* 2014). Oils that initially had a low content of TFAs, repeated heating (e.g. in cooking, deep-frying) can form additional TFAs (Dhaka *et al.,* 2011). TFAs are considered the worst type of fat to eat (Wang *et al.,* 2016). They tend to raise "bad" cholesterol while lowers our "good" cholesterol (Mozaffarian *et al.,* 2010; Wu *et al.,* 2017). Diet high in TFAs increases risk of cardio-vascular diseases (CVD) by 21% and deaths by 28% (Ginter and Simko, 2016).

Given TFAs' adverse health effects, WHO recommends limiting mean population TFAs intake to less than 1% of total energy (%E) (Li *et al.,* 2019). TFAs have no known health benefits and can be replaced in foods without impacting their consistency or taste (Ghebreyesus & Frieden, 2018; WHO, 2018). PHOs are the major source of fats/oils in Tanzania by about 60%, in 2016 it was estimated that, its importation reached about 500,000 MT (3ADI+, 2019), hence the annual consumption of such volumes of dietary source of fat could be posing health threat especially CVDs to consumers (Kagiono *et al.,* 2018). However, there is still limited information regarding the levels of TFAs and their underlying health effects to the Tanzanian population (Codex Alimentarius Commission, 2017). The information generated through this study has shed light on presence of TFAs and its concentrations in some processed commonly consumed foods in Morogoro region.

Materials and methods

Study Area

This study was conducted in Morogoro but in particular Morogoro urban and rural districts (Morogoro region; 6°, 49'S, 37°, 40'E). Morogoro region was selected for the study because previous studies have reported that, it is among the three regions (including Dar es Salaam and Kilimanjaro) which demonstrated a considerably high risk of dying from non-communicable diseases (NCDs) during adulthood in Tanzania (Mayige and Kagaruki., 2013).

Study design

Cross sectional design was used in this study. Samples for TFAs analysis were drawn from two sampling points of each street/village, that is, the street food vendors and restaurants. Four Streets were selected, two from each of the two wards of Morogoro urban and two villages from one ward of Morogoro rural district.

Sampling procedure and sample collection

Simple random sampling was employed in selecting two districts out of 8 in Morogoro region (urban and rural). Furthermore purposive sampling was employed in selecting 2 wards (Mji Mkuu and Mazimbu) out of 29 from Morogoro urban district, and 1 ward (Kiroka) out of 31wards from Morogoro rural district (Mazimbu and Kiroka wards were the most populated wards in their respective districts, while Mji Mkuu ward was selected because it was situated at the town centre). Thereafter simple random sampling was employed to get locations (streets/villages) whereby; 4 (Karume A, Uhuru, Darajani and Boma A) out of 14 streets were selected from 2 wards in Morogoro urban and 2 (Kiroka and Kiziwa) out 4 villages were selected from 1 ward in Morogoro rural district. After that two samples were purposively selected from 2 points (food vendors and restaurants) in each street. Food samples collected included potato chips, wheat buns, "chapatti", rice buns and partially hydrogenated vegetable (PHOs) cooking oil (that had been used for frying chips). During sample collection, forks were used to pick chips, buns, "chapatti" and rice cakes which were then wrapped independently into Aluminium foils while the used vegetable cooking oils were stored in clean amber coloured plastic bottles with their caps tightly closed. All samples were stored in an insulated cool box maintained at a temperature of around 4ºC. They were transported to Food Science Laboratory at Sokoine University of Agriculture the same day and frozen at -18ºC until preparation and extraction.

Materials

Materials used for this study were food samples (wheat buns, "Chapatti", potato chips, rice buns and cooking oil (PHOs) collected from the street food vendors and restaurants. Chemicals (Sulphuric acid 98%, Petroleum ether, Aluminium Chloride hexadrate, Methanol 99.8%, hexane and dichloromethane) all purchased from Loba Chemie Pvt. Ltd, Mumbai (India), and the standard used was Supelco 37 component FAME mix from Sigma-Aldrich. All solvents and reagents were of analytical grade, double distilled water was also used for rinsing. Equipment used included Soxhlet extractor (model Foss Soxtec2055 – made in Germany), GC-MS (model QP 2010Ultra – Shimadzu Japan) Oven (Wagtech–Britain), Restek-5MS column (30m x 0.25mm x 0.25µm), Analytical

Table 1: Number of samples collected from each street/village

Sampling sites	Food Vendors						Restaurants			Total
	Chips	Chapatti	Wheat buns	Rice buns	Used Veg. Oils(PHOs)	Chips	Chapatti	Wheat buns	Rice buns	Used Veg. Oils(PHOs)	
Karume A	1	1	1	1	1	1	1	1	1	1	10
Uhuru	1	1	1	1	1	1	1	1	1	1	10
Mazimbu Darajani	1	1	1	1	1	1	1	1	1	1	10
Boma A	1	1	1	1	1	1	1	1	1	1	10
Kiroka	1	1	1	1	1	1	1	1	1	1	10
Kiziwa	1	1	1	1	1	1	1	1	1	1	10
Total	**6**	**6**	**6**	**6**	**6**	**6**	**6**	**6**	**6**	**6**	**60**

balance (model T5Boeco –Germany), digital food Thermometer (model LDT-3305-China), No 41 Whatman filter papers, Stainless steel fork, Aluminium foils, amber coloured plastic containers and glass vials, Volumetric flasks, Petri dishes, Separating funnels, Ice packs, Cool box were also used.

Sample preparation

Fat and Fatty Acid Methyl Esters (FAME) extraction were carried out by Soxhlet extraction method (Olubunmi *et al.,* 2015). The extracts were packed in an insulated cool box (with ice packs maintained at 4ºC) and then transported to the Department of Fisheries and Aquatic Sciences Laboratory (University of Dar es Salaam, Tanzania) for *trans* fatty acids analysis.

Analysis of Fatty Acids Methyl Ester (FAME) by GC-MS

Analysis of FAME was conducted by Gas Chromatography-Mass Spectrometry. GC-MS were recorded in a GCMS-QP 2010Ultra (Shimadzu, Japan) working in Electron Ionization (EI) mode (MS) at 70ev, and FID for GC. A Restek-5MS column (30m x 0.25mm x 0.25µm) was used. The oven temperature program set at 60ºC to 280ºC, and held at 60ºC for 2 minutes. The temperature was raised to 260ºC for 2 minutes (holding time) at the rate of 13ºC per minute. The injection temperature was 250 ºC with split injection mode. The flow rate of carrier gas (Helium) was 1.21mL min-1. The ion source temperature and interface temperature in MS were 230ºC and 300ºC respectively. Identification of FAME and other compounds in the sample was done by using a scan method which involved the use of Mass Spectral Library and Search Software (NIST). Quantification of FAME in samples was done by using the peak integration method in which ion allowance was 20%, target ion and other 5 quantification ions were employed on quantitative analysis adopting an official method (AOAC 996.06). One (1.0) µL of the sample in dichloromethane was injected in GC MS. The results reported were derived from calibration curve of FAME 37 Mix standard (Supelco37 component, Germany) using peak integration method. To get the concentrations of TFAs, peak

integration was done for individual *trans* fatty acids contents:-

X=(Y+Intercept)/Slope (Khan *et al.,* 2017).

Where by:

X = TFAs concentration (mg/mL) and

Y = Peak Area

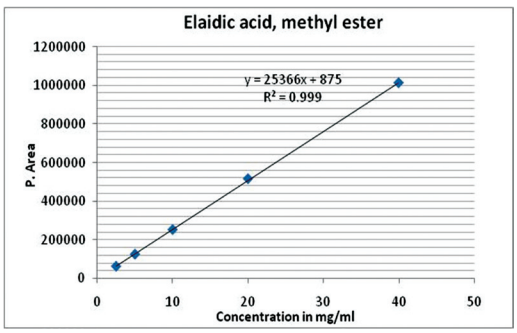

Figure 2: The Calibration curve for Elaidic acid, methyl ester

i. X=(Y- 875)/25366…….....Elaidic acid

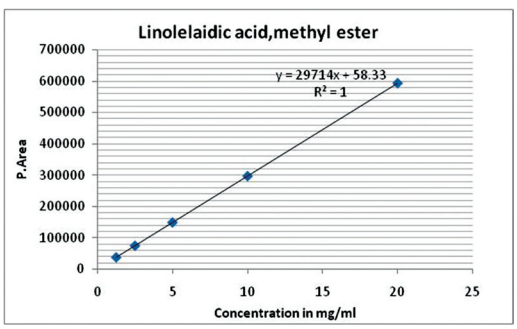

Figure 3: The Calibration curve for Linolelaidic acid, methyl ester

ii. X=(Y-58.33)/29714……...Linolelaidic acid

Statistical analysis

IBM-SPSS statistics Windows software (IBM- SPSS Inc., Chicago, USA; version 20.0) was used for statistical analysis. Descriptive statistics were calculated for the TFAs concentration in g/100g fat. The tool for data analysis was ANOVA & Tukey's HSD whereby a two way ANOVA was used to compare variability in TFAs concentration among food type and location. To check further differences in means for the variables, the Tukey's HSD test (P≤0.05) was performed (Shah *et al.,* 2016) using the following model:-

$$Y_{ijk} = \mu + \alpha_i + \beta_j + (\alpha\beta)_{ij} + \varepsilon_{ijk} \quad (1)$$

Where by:

Y_{ijk} = Dependant variable (TFAs),

μ= Overall mean,

α_i = 1, 2, 3, 4, 5 (Food type effect),

β_j = 1, 2, 3,4,5,6 (location effect),

$(\alpha\beta)_{ij}$ = Interaction effect,

ε_{ijk} = Random error term and k = Replication.

Results

***Trans* fatty acids in processed foods**

Preliminary market survey conducted in the study area identified 5 types of foodstuffs ("chapatti", wheat buns, potato chips, rice buns and used PHOs). These were purposively selected based on the fact that they were all prepared by using partially hydrogenated oils and contained only artificial TFAs.

All analysed food samples contained TFAs with an overall mean of 2.81±1.23g/100g fat, which ranged from 1.80±0.40 g/100g in chapatti to 4.77±0.52 g/100g fat in fried potato chips among ready to eat snacks (Table 2).

The mean TFAs concentration differed significantly (P=0.001) between the food types. However post hoc analysis by Tukey's HSD at P≤0.05, revealed no significance difference in TFAs concentration among wheat buns and rice buns. Furthermore the significant differences were observed between chapatti, potato chips, wheat buns and rice buns while wheat and rice buns showed no significant different.

With regards to the 6 locations, the mean TFAs concentration ranged from 2.54±1.3 g/100g to 3.23±1.47 g/100g fat (with an overall mean of 2.81±1.23 g/100g fat) among the six (6) locations (streets/villages). Furthermore, the overall mean TFAs concentration based on districts were 2.81±1.23 g/100g fat. No significant differences observed with respect to sample's location (either districts or streets) (Table 2). Also no significant differences observed with respect to interaction effects between food samples and locations.

***Trans* fatty acids in Partially hydrogenated oils (PHOs)**

Figure 1 TFAs analysed for PHOs samples from all locations which ranged between

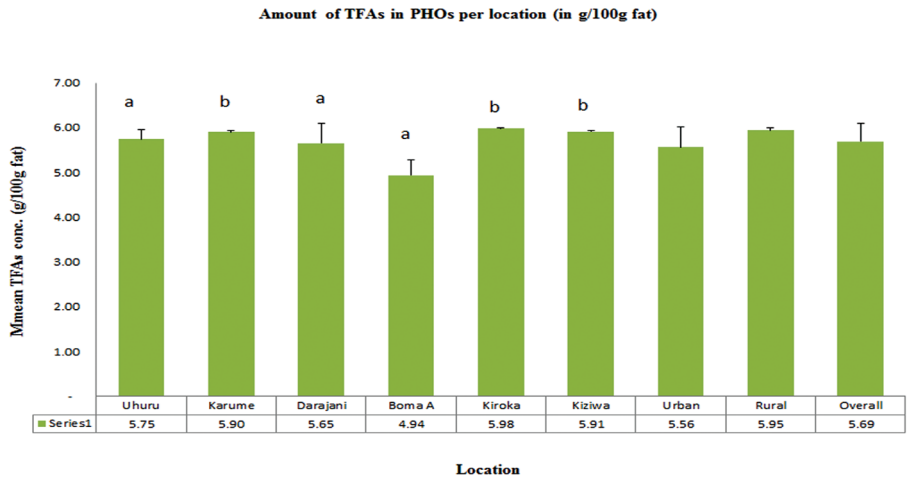

Figure 1: Amount of TFAs in Partially hydrogenated oils (PHOs) based on locations

NB: The locations in bars not sharing a common letter are significantly different (P≤0.05)

Table 2: *Trans* fatty acids in processed food (in g/100g Fat)

Category (Food Type)	**n**	**Mean± SD (g/100g)**	**F-ratio (P- value)**
"Chapatti"	12	1.80±0.40[a]	125.108(0.001**)
Wheat buns	12	2.28±0.38[b]	
Rice buns	12	2.39±0.33[b]	
Chips	12	4.77±0.52[c]	
Overall	48	2.81±1.23	
(Streets/Villages)			
Uhuru	08	3.23±1.47[a]	0.273 (0.925)
Karume	08	2.67±0.93[a]	
Darajani	08	2.84±1.28[a]	
Boma A	08	2.54±1.30[a]	
Kiroka	08	2.87±1.51[a]	
Kiziwa	08	2.71±1.12[a]	
Overall	48	2.81±1.23	
(Districts)			
Urban	32	2.82±1.23[a]	0.007 (0.934)
Rural	16	2.79±1.29[a]	
Overall	48	2.81±1.23	
(Food Type* Location)			1.245 (0.306)

N.B: Given values are means of duplicate analysis along with standard deviation
Means sharing the same superscripts letters along the same column are not significantly different at 5% level of significance.
**Significant results at 5% level of significance.
Food type* Location = Interaction effect of Food type & Location

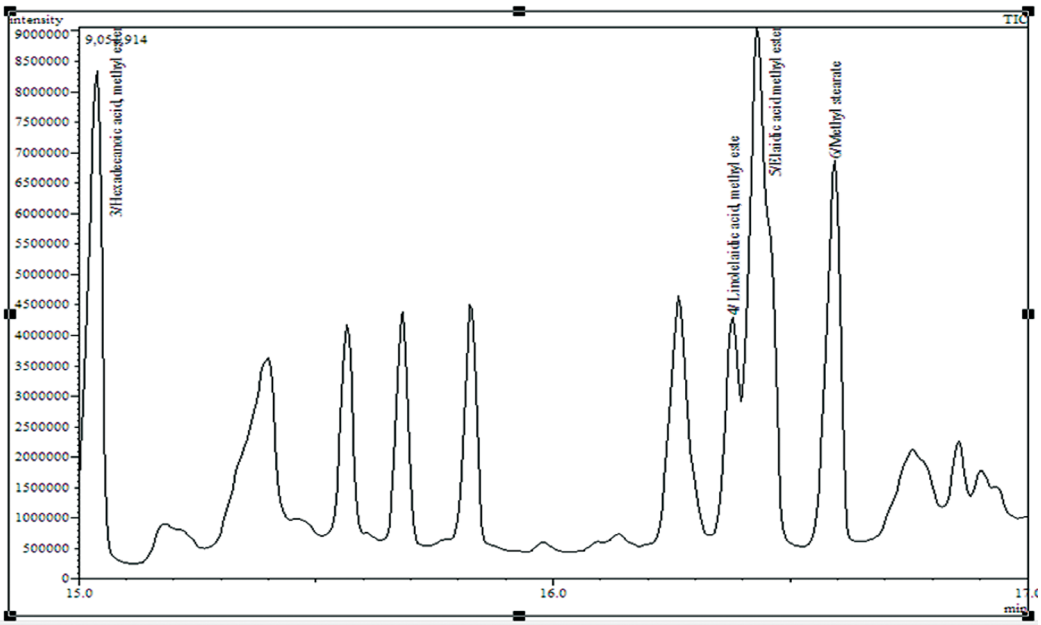

Figure 4: GC Chromatogram of TFAs standard (FAME 37 Mix standard /Supelco37 component)

4.94±0.34 g/100g and 5.98±0.01 g/100g), with an overall mean of 5.69±0.41g/100g fat. TFAs analysis in fresh PHOs (reference oil) was found to contain 4.71±0.04 g/100g fat. Further results in Figure 1 indicate that; generally there was a significance difference in mean TFAs concentration between locations based on streets (F–ratio=5.155; P=0.035). It was confirmed that; PHOs samples from Uhuru Street showed significant difference but was not statistically different from Mazimbu Darajani and Boma 'A' except Karume 'A', Kiroka and Kiziwa (Fig. 1). Additionally, more results of TFAs concentration in PHOs based on districts were 5.56±0.45 g/100g and 5.95±0.04 g/100g fat in urban and rural respectively.

The overall results on the effect of locations based on urban and rural locations on mean TFAs concentration for PHOs, no significant difference was observed (F–ratio=2.75; P=0.128).

Discussion

The overall prevalence indicated that all samples from all locations contained TFAs, although in varying concentrations. Previous studies (Kagiono *et al.,* 2018) have indicated that, TFAs cause lipid and other metabolic disturbances in epidemiological studies, and are consistently linked with a higher risk of cardiovascular disease (CVD) mortality. Data from some countries suggest that TFAs intake may also vary within populations (Wu *et al.,* 2017).

The obtained results of the TFAs in the current study revealed that; the lowest amount (1.80±0.40g/100g fat) was found in "Chapatti". The highest amount (5.69±0.41 g/100g fat) was found in partially hydrogenated oils (PHOs) and the mean was found to be 4.71±0.04g/100g fat. The observed high concentration of TFAs in used PHOs compared to fresh PHOs could be attributed by high heat treatment and repeated heating during chips frying. This argument was also found to be comparable with other studies conducted in Japan, Korea and Palestine (Tsuzuki *et al.,* 2010; Song *et al.,* 2015; Afaneh *et al.,* 2017). Moreover the observed high amount of TFAs in rural area (Fig. 1) could be due to low social economic status compared to urban dwellers hence a higher possibility of re-using cooking oils for several times/days, which leads to further exacerbate the TFAs levels in foods.

A study done in India by Khan *et al.,* (2017) found the amount of TFAs in PHO ranged from 3.31g/100g - 4.73g/100g of fat. Other contrasting findings of TFAs were observed from a study by

Costa *et al.,* (2016) from Portugal which found TFAs levels between 0.26g/100g to 2.16g/100g of fat in PHOs.

Results of the amount of TFAs in the current study reported for cereal products (Chapatti, Wheat and Rice buns) were far lower compared to those reported by Shah *et al.,* (2016) in Pakistan, where it was found to range from 6.190±3.08g/100g fat to 38.691±0.133g/100g fat. Furthermore the current results were comparable to those reported from the survey conducted in Ireland (FSAI, 2016), for the TFAs content in some processed foods (snacks and readymade pastry) which were found to range from ≤0.5 to 4g/100 g fat. Other similar results were previously reported by Costa *et al.,* (2016) in fast foods f ranging from 0.38g/100g to 3.07g/100g fat. Pérez-Farinós *et al.,* (2016) in Spain; found most of food stuffs studied had a TFAs content less than 2g/100g of total fat. The lower amount of TFAs in Spain compared to the current study could be attributed by the strict measures by the Spanish government in reducing TFAs since the ill health effects became known to public.

The levels of *trans* fatty acids in Potato chips revealed by this study were higher (4.77±0.52g/100g) and statistically significantly different from other studied snacks (F-ratio= 125.108; P=0.001). The observed differences could be attributed to high heating temperature, long heating time and repeated heating applications during chips preparations in cooking oils (PHOs) especially in commercial food preparations both in rural and urban locations.

The obtained results in chips were in contrast with those reported from other countries; for instance in India where potato chips from retailers were found to have TFAs level of 2.61g/100g of total fats (Khan *et al.,* 2017), also potato chips and French fries found to contain TFAs in a range from 0.17g/100g to1.26g/100g of total fat in Portugal (Costa *et al.,* 2016; Bloks, 2019). With the exception of "*Chapatti*" with TFAs concentration of 1.80±0.40g/100g fat, the amount of TFAs found in all other foodstuffs were above the optimal recommended amount for TFAs by WHO which is ≤ 2g/100g fat.

It is worthy to note that the variations reported from previous studies, and those of the current study are probably due to the fact that in Tanzania, the policies have not yet enforced to regulate the amount of TFAs in food products especially the iTFAs (Downs *et al.,* 2017; Li *et al.,* 2019) which are regarded as major source of TFAs in foods (Mouratidou *et al.,* 2014; De Jong, 2016). The most effective option to eliminate industrially produced TFAs from our food supply is by implementing regulatory actions to prohibit/ limit their use in foods.

Conclusion

With exception of "chapatti", all samples analysed exceeded the maximum recommended TFAs levels (≤2g/100g fat) as set by the WHO and European Food Safety Authority (EFSA). Consumption of high levels of TFAs from PHOs is a major risk factor for the development of cardiovascular disease (CVD). Tanzania needs to join other countries and impose serious measures to reduce the intake of TFAs in foods. The most effective and consistent way to eliminate industrially produced TFAs from our food supply is by implementing regulatory actions to limit their use in foods. Also public awareness about the ill health effects posed by consumption of foods containing TFAs could be of major help.

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