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# ASSESSMENT OF TRANS FATTY ACIDS IN BAKERY BISCUITS AVAILABLE IN DHAKA, BANGLADESH

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***Abstract***
*Intake of trans fatty acids have adverse health effects like heart disease and information of their contents would enable consumers to make better food choices. Twelve samples of bakery biscuits had been collected in random basis from local markets of Dhaka, Bangladesh to find out the quality and presence of trans fats and toxic elements. For this purpose, Fourier Transform Infrared (FT-IR) analysis and Gas Chromatography-Mass Spectroscopy (GC-MS) analysis of fatty oils extracted from biscuits samples have been performed. Energy dispersive X-ray fluorescence (ED-XRF) analysis was set down to investigate the presence of toxic elements. The ash content, moisture content, acid value, Iodine value, and saponification value were ranged from 0.03-1.55%, 1.71-7%, 1.05-4.68, 32.77-107.91 and 115.19-232.02 respectively. Most of the samples were containing higher percentage of harmful trans fatty acid Elaidic acid (EA). Percentage of the toxic metal Cd, in some sample was found beyond permissible limit (1.0 mg/kg). Biscuit quality and profiling of TFAs constituents provide information on types of partially hydrogenated oils used for the preparation of biscuits, which is important for product reformulation to eliminate TFAs in biscuits. Presence of different fatty acids indicates that palm oil, coconut oil and animal fats were also used for all types of biscuit preparation.*

***Keywords:*** *Bakery biscuits, Fatty oil, Trans fats, Toxic elements.*

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## 1. INTRODUCTION

Biscuit is an important item and now become a common item of consumption among all classes of people. Biscuit is popular because it is nutritious, easy to digest and can be preserved for a long time. Fat forms one of the basic components of a biscuits formulation and is present at relatively high levels. Fat acts as a lubricant and contributes to the plasticity of the cookie dough (Maache-Rezzoug et al. 1998). Fat imparts desirable eating qualities and contributes to texture and flavour of the product. The addition of fat is done principally to stabilize air cells that are generated by mixing (Given 1994). Fat influences the dough machinability during processing, the dough spread after cutting out, and textural and gustatory qualities of the biscuits after baking (Vettern 1984). It interacts with other ingredients to develop and mould texture, mouth feel and overall sensation of smoothness of the product and also influences the rheological properties of cookie dough (Mamat et al 2012). Thermal treatments of fats and oils such as deodorization, cooking, frying etc., generate TFA isomers with limited double bond migration along the carbon chain ( Żbikowska 2010). Some researchers have indicated that serum myristic acid and palmitic acid (Crowe et al 2008) and dietary myristic and palmitic (Kurahashi et al 2008, Männistö et al 2003) saturated fatty acids and serum palmitic combined with alpha-tocopherol supplementation are associated with increased risk of prostate cancer in a dose-dependent manner. Bone mineral density is negatively associated with saturated fat intake, and that men may be particularly vulnerable (Corwin et al 2006). Effect of some specific saturated fatty acids is described as follows Myristic acid raises LDL cholesterol and decreases HDL cholesterol. Pentadecylic acid is responsible for mother to child transmission of HIV through breastfeeding. Heptadecanoic acid is responsible for myocardial infarction in

women. Stearic acid does not raise LDL cholesterol and HDL cholesterol level. Lauric acid has a more favorable effect on total/HDL cholesterol than any other fatty acid either saturated or unsaturated; (Mensink et al 2003) a lower total/HDL cholesterol ratio suggests a decrease in atherosclerotic risk (Thijssen and Mensink 2005). Caprylic capric acid decreases LDL cholesterol and used in treatment crohn's disease, and bacterial Infections. Inthese saturated acids myristic, palmitic,pentadecylic and heptadecanoic acids are harmful to health. But lauric, stearic, caorylic and capric acids are useful to health.

Elaidic acid is the principal trans fatty acid often found in partially hydrogenated vegetable oils. It has a much higher melting point, 45 °C than oleic acid, 13.4 °C and remains in solid form at human body temperatures (Hill and Kolb 2007). The intake of both trans fats and saturated fats promote the development of Alzheimer disease (Ascherio et al 1999). It has been found a positive connection between trans fat and prostate cancer (Phivilay et al 2009). An increased intake of trans fatty acids may raise the risk of breast cancer by 75% (Orge et al 2006). The risk of type 2 diabetes increases with trans fat consumption for those in the highest quartile of trans fat consumption (Brasky et al 2011). Research indicates that trans fat increase weight gain and abdominal fat, despite a similar caloric intake (Chajès et al 2008).

Heavy metal toxicity can result in damaged or reduced mental and central nervous function, lower energy levels, and damage to blood composition, lungs, kidneys, liver, and other vital organs. Some of the heavy metals are necessary for humans in minute amounts while others are carcinogenic or toxic, affecting, , the central nervous system , the kidneys or liver , skin, bones, or teeth (Duffus 2002).

Ingestion of heavy metals through foods is hazardous and data on contamination of foods is not available for many developing countries including Bangladesh. To assess the risk to human's health arising from the presence of Trans fat and heavy metal in foods, the actual dietary intake of metal should be estimated. In view of this fact, the present study presents an account of presence of trans fat and heavy metal in some brands of biscuits in Bangladesh.

## 2. MATERIALS AND METHODS

Moisture, total ash content were performed according to AOAC official methods 925.09 and 923.03 respectively Official Methods of Analysis of AOAC (2003) International. Chemical properties of oil namely iodine value, saponification value, acid value were determined according to AOAC official methods 28.023 – 1984, 28.026- 1984 and 996.01- 1984 respectively (AOAC 2003).

***Extraction of fatty oil from biscuits samples:***

The fatty oil sample was extracted by using extraction method by using 40- $60^0$ C. pet ether as solvent from powdered biscuits samples in a flat bottom glass Soxhlet apparatus as following. Definite amount of moisture free powdered biscuits samples were taken in a thimble, which was prepared by filter paper. Then the thimble was placed in the Soxhlet apparatus unit and extraction was carried out with 40°- 60° c. petroleum ether for 6 hrs. on a heater at 80°C-90°C. The fatty oil in the solvent obtained from the soxhlet unit was filtered to remove impure materials. The solvent was removed using rotary evaporator vacuum pump. Then fatty oil was dried in an oven at 110°C. And cooled in desiccators and weighed. By this process the purified fatty oil was obtained for further characterization.

***Fatty Oil Analyses by Gas Chromatography and Mass Spectrometer (GC-MS):***

Non-reactive derivatives of fatty acids (methyl esters or other derivatives) was prepared before GC-MS analysis. An aliquot of lipid extract (about 10 mg) was dried under nitrogen in a screw-capped glass tube and 1 ml of $BF_3$/methanol (14%) was added. If triacylglycerols or sterol esters were analyzed in the extract, the dry lipids are dissolved in 0.75 ml of chloroform/methanol (1/1, v/v) and 0.25 ml $BF_3$/methanol are added. After cooling, 1 ml water and 2 ml pentane add. GC model 7890A Agilent Technologies was used. 1 micro liter sample was injected in split less mood. The inlet temperature was 260°C and oven temperature was 70°C (0 min.); 10°C, 150°C (5 min.); 12°C, 200°C (15 min.); 12°C,

220°C, (5 min.). Total run time was 38.833 min and column flow rate 0.6 ml/min He gas. The GC_MS interface temperature was 280°C. The MS (Model : Agilent Technologies 5975C inert XL EI/CI MSD with triple Axis Detector) was set in scan mood. The ionization mode was EI type . The mass range was 50-550 m/z. MS Quad temperature was 150°C and MS Source temperature was 230°C. The fatty oils of Biscuits samples were analysis by Electron Impact ehavior on (El) method on GCMSD; fused silica capillary column (30m x 0.25 mm). The fatty oil peaks were compared with peaks of reference by overlapping the chromatogram.

***IR Analysis:*** FTIR (IR Prestige-21), Shimadzu, Japan was used for the analysis of the fatty oil extracted from the biscuit samples. 5uL of fatty oil dropped in between two discs of KBr and placed in the spectrophotometer by a holder.

***Elemental Analysis:*** The elemental analysis of powdered biscuits was done by Energy dispersive X-ray Fluorescence (Quantx EDXRF Analyzer C10020, USA).

## 3. RESULTS AND DISCUSSION

The ash content, Organic matter, moisture and dry matter are presented in Table-1. Permissible percentage of ash was 1% for Biscuits recommended by "Indian Standards Institution" (ISI). The values of ash contents of Biscuits determined were presented in Table -1. Ash content had found in only one sample viz., sample S12 higher than ISI recommended value. Percentage of ash in Sample S2 was 0.03 and Sample S4 was 0.04 which were very low. These two samples contained low amount of minerals. Percentage of ash in Sample S8 was 0.34. The mean values of moisture content in Biscuits determined were presented in Table-1. Moisture significantly affect on shelf life and growth of the microbes. The lower the moisture contents of products, the better the self stability of the product. The maximum limit of moisture content is 5.0% recommended by Bangladesh Standards and Testing Institution (BSTI) for biscuits (BDS383 2001). The result showed that the moisture content of biscuits were within the range of 5-9%. Percentage of moisture in Sample S10 had the lowest moisture content of 1.71±0.01% while S3 had the highest moisture content of 8.28±0.037%. This means that biscuits sold in the market of Bangladesh meets up to standard in terms of moisture content and that they can be stored for a period of time prior to consumption when properly packed from moisture.

Results of acid value, iodine value and saponification value of fatty oil of biscuits samples are given in Table-2. Acid value of fatty oil for all samples were found beyond the permissible limit 1.0 recommended by BSTI for biscuits. It was found to increase irregularly up to 4.7 times of permissible limit. Fatty oil of S6, S9 and S10 samples contained minimum amount of free acids. But acid values for others samples were twice or 4.7 times higher than accepted limit.

Iodine value is the measure of unsaturation of fatty oil. Result shows that iodine value of S9 was lowest and S12 was highest. Among others for S1, S3, S6, S7 iodine values were comparatively lower than sample S2, S4, S5, S8, and S10. For these samples it was found to increase irregularly up to three times of permissible limit. Accepted level of iodine value was 26-35 recommended by BSTI for butter and ghee. Only S9 sample was found with in the range (32.77). That means fatty oils of all samples were highly unsaturated.

Saponification value was a measure of the average molecular weight (or chain length) of all the fatty acids. The permissible limit of saponification value is 218 recommended by BSTI for butter and ghee. It was found fatty oils of samples S8, S9 and S11 have higher saponification values than permissible limit. But these values were very close to the permissible limit. Percentage of short chain fatty acids will be slightly more in these samples as compared to the other samples. From the experimental result it was reported that all samples had long chain fatty acids and relatively fewer number of carboxylic functional groups per unit mass of the fats. Result showed that saponification value was lowest for S12 and highest for S11.

Fatty oil analysis of all the samples showed (Table 3) that they contained trans fatty acid

viz. Eladic acid which was responsible for cardiovascular diseases. Beside this the samples contained harmful saturated fatty acid viz. Palmitic acid, Myristic acid, which increases LDL cholesterol level and decreases HDL cholesterol level in blood. Saturated fatty acid viz. stearic acis was found to be associated with lowered LDL cholesterol and was not threat for health in broader sense. On the other hand very helpful monounsaturated fatty acid viz. oleic acid and essential fatty acids viz. linoleic acid and lauric acid were present in small amount.

Fatty oil obtained from Biscuit sample S7 contain of 7-methoxy-2,2-dimethyl-6-vinyl-2H-cromene, 1-chloro-3-methoxy-1, 3-dimethyldisiloxane and 2-isopropyl-3-methoxycyclopropanecarboxylic acid were also found. Fatty oil obtained from Biscuit S8 contained of 6-methoxy-2, 3, 4, 4a, 5, 10-hexahydrobenzo[g] quinolinium chloride was found. Fatty oil obtained from Biscuit S9 contained Caprylic and Capric acids. The IR analysis confirms the functional groups of the fatty oil components (Table 4).

Dietary trans fatty acids are transformed by the placenta to the foetus and incorporate into foetal tissues. According to Koletzko and Desci (1997) TFAs may contribute to infantile birth weight in preterm and health term babies as well as reduce the duration of pregnancy. Elaidic acid (18:1, trans-9) is the major man-made transfat found in hydrogenated vegetable oils and processed foods (Craig-Schmidt MC 1992).

Low amounts of trans fatty acids occur naturally in animal products, such as beef, lamb and dairy products and Elaidic acid (C18:1t9) typically is the major isomer in industrial sources of TFAs. The C18:1t isomers constitute approximately 80-90% of the total TFA in foods. Other isomers including C16:1t, C18:2t, C18:3t, and long chain polyunsaturated TFA can also be an important component of the total TFAs (Weggemans et al.,2004; Kraft et al.,2006; Stender et al., 2006). In IR analysis (Table-4, Fig:1) same functional groups were obtained compared to the compounds obtained by GC-MS analysis (Fig:2).

Elemental composition of biscuits samples by EDXRF analysis was performed for the screening out toxic elements (Table 5). Results obtained from the analysis were presented in the table 5 in m. Result showed there was no toxic element in samples except sample S3 where element cadmium (0.245% m/m) was present.

The permissible level of cadmium in foods was 0.05 µg/g (FAO/WHO, 2001). Others elements were not harmful to health. some individual samples contained cadmium at levels above the permissible limit.

**Table 1: Parameters of biscuits**

| Sample ID | % of Ash | % of Organic matter | Moisture (%) | Dry matter (%) |
|---|---|---|---|---|
| S1 | 0.52 | 99.47 | 5.17±0.071 | 94.82±0.07 |
| S2 | 0.03 | 99.96 | 5.95±0.037 | 94.04±0.038 |
| S3 | 0.79 | 99.20 | 8.28±0.012 | 91.71±0.013 |
| S4 | 0.04 | 99.95 | 4.24±0.19 | 95.75±0.193 |
| S5 | 0.55 | 99.45 | 3.15±0.004 | 96.84±0.008 |
| S6 | 0.54 | 99.46 | 3.77±0.006 | 96.22±0.01 |
| S7 | 0.66 | 99.34 | 3.99±0.19 | 96.01±0.14 |
| S8 | 0.34 | 99.66 | 2.33±0.058 | 97.67±0.01 |
| S9 | 0.88 | 99.11 | 3.08±0.11 | 96.91±0.32 |
| S10 | 0.59 | 99.40 | 1.71±0.014 | 98.28±0.019 |
| S11 | 0.75 | 99.24 | 4.84±0.011 | 95.15±0.06 |
| S12 | 1.55 | 98.44 | 7.00±0.012 | 92.99±0.12 |

**Table 2: Characteristics of oil extracted from the biscuits**

| Sample ID | Acid Value | Iodine Value | Saponification Value |
|---|---|---|---|
| S1 | 2.29±0.15 | 42.34±1.19 | 143.16±4.93 |
| S2 | 4.32±0.21 | 60.60±2.17 | 181.31±2.84 |
| S3 | 2.24±0.11 | 42.64±1.19 | 147.27±0.98 |
| S4 | 2.20±0.05 | 57.30±0.81 | 174.56±1.33 |
| S5 | 3.12±0.02 | 61.42±1.25 | 217.34±2.68 |
| S6 | 1.05±0.06 | 51.77±1.18 | 187.99±3.93 |
| S7 | 2.29±0.03 | 46.30±1.19 | 184.39±4.75 |
| S8 | 4.08±0.12 | 74.02±1.52 | 220.51±1.42 |
| S9 | 1.10±0.02 | 32.77±1.17 | 226.81±5.32 |
| S10 | 1.11±0.08 | 61.55±1.16 | 191.51±2.31 |
| S11 | 3.43±0.12 | 55.65±1.39 | 232.02±2.16 |
| S12 | 4.68±0.08 | 107.91±3.21 | 115.19±1.42 |

**Table 3: Fatty acid composition of the oil extracted from biscuits**

| | S1 | S2 | S3 | S4 | S5 | S6 | S7 | S8 | S9 | S10 | S11 | S12 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Caprylic acid | - | - | - | - | - | - | - | - | 5.65 | - | 0.74 | 6.63 |
| Capric acid | - | - | - | - | - | - | - | - | 4.28 | - | 1.49 | 5.14 |
| Lauric acid | 0.88 | 1.51 | - | 1.01 | - | 4.12 | - | - | 21.27 | - | 2.31 | 24.65 |
| Myristic Acid | 3.05 | 2.05 | 1.52 | 3.17 | 1.72 | 3.06 | 1.32 | 1.45 | 9.21 | 1.75 | 5.45 | 10.71 |
| Palmitic acid | 36.95 | 29.42 | 33.02 | 33.99 | 33.12 | 32.45 | 36.95 | 33.41 | 22.99 | 35.61 | - | 17.84 |
| Heptadecanoic acid | - | - | 0.88 | - | 0.99 | - | - | - | - | - | - | - |
| Stearic acid | 8.55 | 6.82 | 4.53 | 8.67 | 4.74 | 6.31 | 4.70 | 5.70 | 4.58 | 6.99 | 10.31 | - |
| Oleic acid | 3.99 | 3.24 | 1.55 | 5.86 | - | - | - | - | - | 31.60 | 2.71 | 4.46 |
| Elaidic acid | 31.20 | 28.53 | 30.64 | 28.66 | 39 | 29.10 | 28.10 | 31.38 | 17.26 | 5.25 | 29.66 | 19.39 |
| Linoleic acid | 9.71 | 20.09 | 13.80 | 8.99 | 14.59 | 9.57 | 10.02 | 10.42 | 7.97 | 13.41 | 4.40 | 6.96 |

*Results are expressed as area%

**Table 4: IR Spectra of fatty oil of S1**

| Peak No. | Frequency ( $cm^{-1}$) | Intensity | Functional group | Compound Type |
|---|---|---|---|---|
| 1 | 720 | bending | C-H | *cis* Alkene |
| 2 | 970 | weak | C-H | *trans* Alkene |
| 3 | 1163.08 | strong | C-O | Ester, Carboxylic acid, Alcohol, Ether, Anhydrides |
| 4 | 1375 | bending | $CH_3$ | Alkane |
| 5 | 1465.90 | strong | $CH_2$ | Alkane |
| 6 | 1616.35 | strong | C=C | Alkene |
| 7 | 1745.58 | strong | C=O | Ester, Carboxylic acid, Aldehyde, Ketone |
| 8 | 2852.72 | strong | C-H | Alkane, |
| 9 | 2922.16 | strong | O-H | Carboxylic acid |
| 10 | 3005 | moderate | C-H | Alkene |

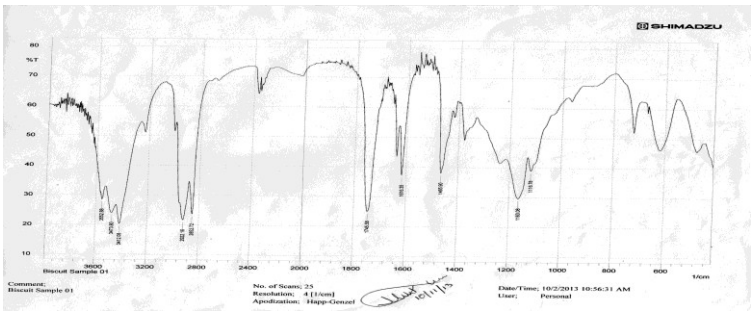


**Fig 1: Representative IR spectra of Fatty oil extracted from Sample S1**

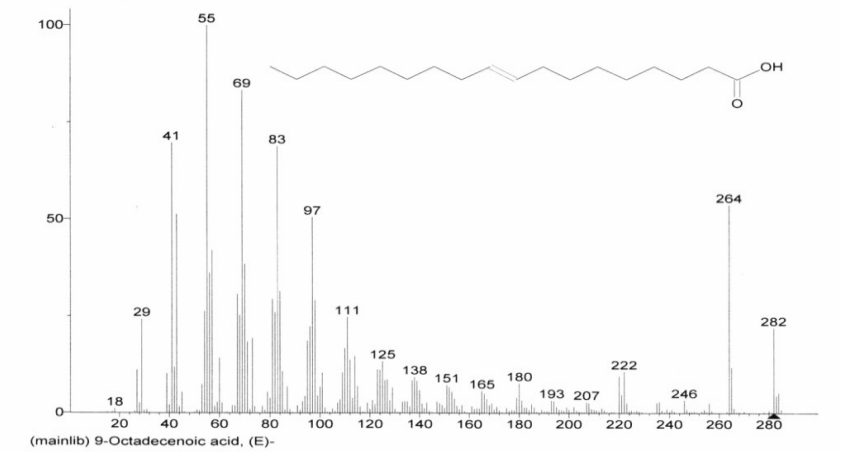


**Fig 2: Mass Spectra of 9-octadecenoic acid (E)- (Elaidic acid)**

**Table 5: Elements in biscuits by EDXRF (m/m%, m=metal)**

| Elements | S1 | S2 | S3 | S4 | S5 | S6 | S7 | S8 | S9 | S10 | S11 | S12 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Ca | 12.23 | 45.08 | 15.20 | 31.42 | 22.16 | 33.84 | 30.80 | 17.82 | 44.99 | 12.57 | 44.97 | 18.21 |
| K | 9.95 | 21.60 | 38.55 | 47.29 | 42.75 | 41.53 | 36.64 | 37.50 | 17.04 | 37.10 | 37.66 | 40.32 |
| Zn | 0.46 | 1.04 | 0.262 | 0.85 | 0.674 | 1.21 | 0.531 | 0.735 | 0.252 | 0.291 | 1.15 | 0.444 |
| Cu | - | 0.75 | 0.163 | - | 0.286 | 0.53 | 0.321 | 0.427 | - | - | 0.12 | 0.215 |
| Mn | - | 0.60 | 0.30 | - | - | - | - | - | - | 0.333 | - | - |
| Ti | - | 0.49 | - | - | - | - | - | 0.68 | 0.238 | - | - | - |
| Cd | 0.245 | - | - | - | - | - | - | - | - | - | - | - |
| Co | - | - | - | 0.25 | 0.180 | - | - | - | - | - | - | - |
| Fe | - | - | - | - | - | - | - | 2.87 | 4.41 | 0.77 | - | - |

Iwegbue (2012) reported the presence of cadmium in biscuits in nigeria. Salama and Radwan, 2005 reported cadmium contents ranging from 0.013-0.122 µg/g in biscuits from Egypt, Karavoltsos *et al*. (2002) reported cadmium contents in biscuits from Greece as 0.0126-0.0143 µg/g. Gopalani *et al*. (2007) found cadmium at levels below the limit of detection in biscuits from India. Cadmium is unique among the metals because of its combination of toxicity in low dosages, long biologic half-life ,its low rate of excretion from the body, and the fact that it is stored predominantly in the soft tissues like liver and kidney (Beliles, 1994).The effects of cadmium on humans are nephrotoxicity, osteotoxicity, cardiovascular-toxicity and effects on reproduction and development and genotoxicity (European Community, 1996). About 5% of ingested cadmium is absorbed (Friberg et al., 1986). The speciation of cadmium in foodstuffs may be of importance for the evaluation of the health hazards associated with areas of cadmium contamination or high cadmium intake (WHO, 1992).

Absence of Trans fatty acid in edible oils available in Bangladesh was confirmed by Chowdhury et al. (2007). So, the source of the elaidic acid was may be due to the use of partially hydrogenated oil for the preparation of biscuits. Presence of Elaidic acid in buiscuits and cookies was confirmed by different studies (Fu et al. 2008). Sample S8, S11 and S12 contains Caprylic, Capric and Lauric acid may be due to the use of coconut oil. Sample S3 and S5 contains Heptadecanoic acid (Margaric) which indicates the use of animal fat for the biscuit preparation (Norhayati et al 2011). Palmitic acid, Linoleic acid, Oleic acid and Stearic acid was found in all the samples as most fats and oils are the source of these fatty acids.

## 4. CONCLUSION

Trans fatty acids contribute to high risk of CVD, developing type 2 DM and cancer. The major source of trans fat in human diet is

bakery product like biscuits and cookies. The result of the present study indicate that biscuits available in Bangladesh contains trans fatty acid elaidic acid along with other saturated and cis unsaturated fatty acids. Some sample contains heavy metals higher than the permissible limit. On the other hand high percentage of saturated fatty acid mainly palmitic acid was obtained and saturated fatty acids were found in small amount. Intake of these biscuits containing TFA for long period will be harmful to health. So manufacturer should avoid partially hydrogenated oil for the preparation of biscuits. Regulatory authorities should take steps to control and determine the limit of trans fat in food. Since the adverse affects of trans fatty acids have been conclusively shown in many studies, analyzing the trans fat in foods in every region are essential to assess the actual risk that consumption of these foods carries rather than relying on the food composition tables.

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