Fatty Acid, Carotenoid and Tocopherol Content of Some Fast Foods from a Nigerian Eatery

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Abstract   The study investigated the quantitative and qualitative [chemical] characteristics of fatty acids, carotenoids and tocopherol in some fast foods prepared from a Nigerian eatery. The fatty acid profile was determined by gas chromatography, carotenoids by open column chromatography and visible spectrophotometry, and tocopherol by colorimetric method. The free fatty acid, acid and peroxide values were carried out by method of analysis of association of official analytical chemist (AOAC). The results showed that palmitic acid is the most prevalent saturated fatty acid while mono unsaturated and poly unsaturated fatty acids ranged from 40 to 46% and 9.5 to 12%, respectively. Trans fatty acid and eicosapentaenoic acid were present in some samples. The oxidation products were higher than acceptable limits. The level of carotenoids was low but the tocopherol content could provide the requirement for vitamin E. Fast foods contain factors that could predispose consumers to non communicable health diseases like coronary health diseases.

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1. Introduction

Fast food culture is now a vogue in Nigeria, and fast food industry is taking advantage of the enthusiasm of Nigerians for this service to expand operations. The first fast food outlet (now with other 170 outlets) in Nigeria was opened in 1986 and the second in 1997. There were 15 such outlets by year 2000 and the list now seemed endless because new businesses enter the market virtually every day. Nigerians who can afford it, embrace the fast food culture as "hip" at a time people in Western countries are being cautioned on the potential negative health effects of high-fat, high-calorie "junk food" [1]. Consumption of fast food has been associated with coronary heart disease (CHD) and other non communicable or non contagious diseases [2] based on the assumption that the fat content could contain factors that could predispose the consumers to some of these health challenges.

Lipids are important in foods due to their contribution to palatability, satiety and nutrition. Therefore, lipid quality is significant to consumers [3]. Heating or cooking by microwave, deep-frying and baking, as employed in fast food preparation, involve high levels of energy. These thermal processes oxidize lipids, especially unsaturated fatty acids, to form conjugates and polymers within minutes since microwave, for example, cause the molecules to vibrate strongly, affecting especially the hydrogen atoms of the active methylene groups which are adjacent to the unsaturated centers [4]. Oil undergoes

three deleterious reactions: hydrolysis caused by water, oxidation caused by oxygen and thermal alteration by heat [5]. These deleterious reactions lead to polymerization and homolytic β-scission of hydro-peroxides.

Decomposition products are also formed as a result of reactions between food ingredients and oil, affecting products' taste, flavour and shelf-life [6]. Oxidation plays a significant role due to the development of rancid flavours that reduce the organoleptic characteristics; and the formation of oxidized products that may cause a health hazard [7].

The quality of oil in foods has an ultimate contribution to the overall nutritional quality of that food [8], and can be estimated through measurement of chemical parameters such as free fatty acid, peroxide value and acid value. Besides, alteration in fatty acids, the content of lipophilic vitamins like vitamin E and pro-vitamin A carotenoids, may be affected. These essential lipophilic nutrients apart from their vitamin activity are well known for their role as chain-breaking antioxidants during lipid peroxidation and for protection of polyunsaturated fatty acids in cell membranes from oxidation.

The fast food industry in Nigeria is young, and information is scanty on the dietary quality of fat as well as the content of lipophilic vitamins in foods prepared by fast food eateries in Nigeria. The objective of this study was therefore to carry out the analysis of fatty acid profile, lipophilic vitamins and quality assessment in terms of chemical composition of oil extracted from fast foods with a view to providing information on the risk status of fast food consumption in Nigeria.

2. Materials and Methods

2.1. Sample Preparation

Samples of fast foods (jollof rice, fried rice, meat pie, doughnut, hot dog, beef roll), as consumed, were collected in food flask from five outlets of a popular fast food eatery located in Lagos, Nigeria. The meals were weighed and dried in the oven (Gallenkamp Oven Model SA 9059 B) at 50°C. The meals were ground into powder; sieved with No 72 mesh size, (Griffin and George Ltd., London) stored in plastic containers with screw cap, and then kept in the freezer until used.

2.2. Extraction of Fat

Lipid from 10.0 g samples was extracted with hexane until the thimble showed no appearance of oil. The solvent was distilled off; the oil was weighed and kept for further analysis. The lipid content was determined by comparing the weight of the extracted oil to the weight of the sample taken.

2.3. Fatty Acid Analysis

The extracted oil was analyzed for fatty acid profile by AOAC method 965.33 [9], using Agilent 6890 gas chromatograph equipped with an on-column automatic injector, flame ionization detector, HP-88 capillary column (100 m x 0.25 µm film thickness; Hewlett-Packard, Sunnyvale, CA, USA) and a Chemstation software (Agilent technologies, U.S.A). The operating conditions were: carrier gas, helium; injector temperature, 250°C, detector temperature, 280°C; temperature program, initially at 175°C, raised to 220°C at a rate 2°C / min, and held at 220°C for 20 min. The fatty methyl esters were identified by comparing their retention times to those of known standards and quantified using the principle of internal standardization. The coefficient of variation for the method was less than 4%.

2.4 Determination of Acid Value and Free Fatty Acid

The acid value and free fatty acid content were determined by AOAC method 940.28 [9]. The oil sample (0.2 g) was dissolved in 10 mL ethanol and titrated with 0.1M NaOH solution using phenolphthalein indicator until pink colour disappeared. The acid value and the percentage fatty acid were calculated from the expression below:

$$Acid\ Value = \frac{56 \times molarity\ of\ NaOH \times titre\ value}{weight\ of\ oil}$$

$$\%\ Free\ Fatty\ Acid\ as\ Oleic\ acid = 0.503 \times Acid\ Value$$

2.5. Determination of Peroxide Value (PV)

The oil sample (5.0 g) was weighed into a 250 ml conical flask, dissolved with 30 mL solvent mixture containing 12 mL chloroform and 18 mL glacial acetic acid. Saturated aqueous potassium iodide solution (0.5 mL) was added; the flask was stoppered and allowed to stand for one minute. Thereafter, 30 mL of distilled water was added and the solution was titrated against 0.1 M sodium thiosulphate solution until the yellow colour had almost disappeared. At this point, starch solution (0.5 mL) was added and the titration continued until the blue-black colour disappeared. The same procedure was carried out for a 'blank' determination, where the oil sample was excluded. The peroxide value was calculated from the expression below:

$$P.V\left(meg/kg\right) = \frac{\left(S-B\right) \times M \times 1000}{weight\ of\ sample(g)}$$

where;
meq/kg = milliequivalent peroxide/kg sample
S = Titre value (mL) of sodium thiosulphate for sample
B = Titre value (mL) of sodium thiosulphate for blank,
M = Molarity of sodium thiosulphate solution

2.6. Determination of Pro-Vitamin a Carotenoids

Carotenoid content of the samples was determined by AOAC method 970.64 [9]. Samples were extracted with cold acetone including 0.005% Butylated hydroxyl toluene (BHT), filtered, pooled and washed three times with petroleum ether (boiling point 60 - 80°C). The petroleum ether extract was washed with distilled water and dried over anhydrous sodium sulphate. The dried petroleum ether extract was concentrated in a rotary evaporator ( ) under vacuum at 25°C, pour into 25 mL standard and made up to mark. The extract (5.0 mL) was chromatographed on a magnesium oxide: Hyflo Supercel (1:2) column (15 mm i.d x 15 cm long). The following fractions were eluted from the column using 10 mL each of the following elution solvents: α – carotene was eluted with 1.0% acetone in petroleum ether, β – carotene with 5.0% acetone in petroleum ether and cryptoxanthin with 10% acetone in petroleum ether. Each of the fractions collected above was placed in a 25 ml standard flask and diluted to volume with petroleum ether. Absorbance of each fraction was scanned between 350 and 530 nm using a spectrophotometer (Pye Unicam Ltd, UK). The concentration of the carotenoids was calculated using the following extinction coefficients: α- carotene (2640); β-carotene (2480); and cryptoxanthin (2460) as described by Klein and Perry [10]. Each carotenoid was converted to international unit [I.U.] (retinol equivalent) by assuming that 0.6 µg β-carotene or 1.2 µg α- carotene and cryptoxanthin was equivalent to 1.0 I.U. (NAS-NRC, 1980) [11].

Carotenoids were calculated using this expression;

$$Garotenoid\left(\mu g/g\right) = \frac{A_{total} \times volume\left(ml\right) \times 10}{E^{1\%}_{1cm} \times sample\ weight}$$

where;
A_{total} = Absorbance of the sample
$E^{1\%}_{1\ cm}$ = Extinction coefficient of the carotenoid

2.7. Determination of Tocopherol

Total tocopherol was determined by AOAC method 971.30 [9]. The sample (5.0g) was washed with a mixture of ethanol – petroleum ether (ratio 2:1) until the residue was devoid of oil, the extracts were pooled together, put into separating funnel and 2.0 mL of ethanolic potassium

hydroxide solution was added to saponify the oil. The mixture was allowed to settle; ethanol layer was decanted off, while the petroleum ether layer was washed to remove soap and dried over anhydrous sodium sulphate. The extract (1.0mL) was put in screw cap text tube, 1.0 mL of bathophenanthroline, 0.5 mL $FeCl_3$ and 0.5 mL H_3PO_4 solutions were added. The absorbance was measured at 534 nm. The standard and the blank were also determined. Tocopherol content was extrapolated from the standard curve and I.U. potency was calculated using the conversion method that 10 I.U. = 4.5 mg α-tocopherol.

3. Statistical Analysis

Analysis was carried out in triplicate for each sample and the results were presented as mean and standard deviation of replicate analysis (n=5) of samples collected for each food type. Analysis of variance (ANOVA) was used to assess and compare results. Statistical analysis was carried out by the use of Microsoft Excel Statistical Packages (Microsoft Corporations, USA) and Graph-Pad Instat-3 Packages (Graph Pad software Inc, USA).

4. Results

The result reported in Table 1 gave the range of saturated fatty acids (SFA) as 42 to 50% of the total fatty acids with palmitic acid (C16:0) predominating followed by stearic acid (C18:0). The result indicated that the oil was high in mono-unsaturated fatty acid (MUFA), consisting mainly of oleic acid. The poly unsaturated fatty acid (PUFA) consisted of linoleic acid (C18:2) and α-linolenic acid (C18:3), while eicosapentaenoic acid (C20:5) was detected only in fried rice. Trans fatty acids were found in doughnut, hotdog and fried rice within a range of 0.05 to 0.17%.

Table 1. Fatty Acid Profile presented as % of the total fatty acids

Sample/Fatty acids	Doughnut	Meat pie	Hotdog	Beef roll	Jollof Rice	Fried Rice
	Saturated fatty acids (SFA)					
C8:0	0.32 ± 0.05^{ab}	0.24 ± 0.03^{b}	ND	0.24 ± 0.02^{b}	ND	0.40 ± 0.07^{a}
C12:0	0.33 ± 0.03^{b}	0.86 ± 0.16^{a}	0.19 ± 0.05^{c}	0.86 ± 0.16^{a}	0.22 ± 0.16^{c}	0.23 ± 0.02^{c}
C14:0	0.94 ± 0.01^{b}	1.31 ± 0.11^{a}	0.87 ± 0.06^{b}	1.31 ± 0.11^{a}	1.02 ± 0.04^{a}	0.84 ± 0.02^{b}
C15:0	0.03 ± 0.05	ND	0.04 ± 0.03	ND	ND	ND
C16:0	38.07 ± 0.5^{b}	42.20 ± 1.1^{a}	40.92 ± 0.32^{a}	42.70 ± 1.10^{a}	35.76 ± 0.31^{c}	34.7 ± 0.4^{c}
C17:0	0.17 ± 0.02^{a}	ND	0.10 ± 0.07^{b}	ND	ND	0.17 ± 0.02^{a}
C18:0	4.52 ± 0.07^{b}	5.11 ± 0.23^{a}	5.28 ± 0.02^{a}	4.51 ± 0.23^{b}	4.98 ± 0.22^{a}	4.3 ± 0.1^{b}
C20:0	0.49 ± 0.02^{a}	0.20 ± 0.28^{b}	0.47 ± 0.01^{a}	0.20 ± 0.28^{b}	0.51 ± 0.09^{a}	0.49 ± 0.02^{a}
C22:0	0.10 ± 0.07	ND	0.10 ± 0.07	ND	ND	0.10 ± 0.08
C24:0	0.09 ± 0.07	ND	0.08 ± 0.06	ND	ND	ND
C26:0	0.03 ± 0.03	ND	ND	ND	ND	ND
Total SFA	45.09 ± 0.1^{b}	49.92 ± 0.1^{a}	48.05 ± 0.05^{a}	48.51 ± 0.1^{a}	42.49 ± 0.05^{c}	41.20 ± 0.04^{c}
	Monounsaturated fatty acids (MUFA)					
C14:1	ND	ND	ND	ND	ND	0.06 ± 0.00
C16:1 cis	0.27 ± 0.06^{c}	0.47 ± 0.02^{b}	0.21 ± 0.03^{c}	0.48 ± 0.01^{b}	1.49 ± 0.06^{a}	0.20 ± 0.09^{c}
C18:1 cis	42.62 ± 0.57^{a}	38.4 ± 0.40^{c}	40.74 ± 0.15^{b}	38.9 ± 0.4^{c}	43.8 ± 0.8^{a}	43.1 ± 0.4^{a}
C20:1 cis	0.20 ± 0.01^{b}	ND	0.12 ± 0.09^{c}	ND	0.26 ± 0.01^{b}	0.4 ± 0.2^{a}
Total MUFA	43.1 ± 0.2^{b}	38.87 ± 0.07^{d}	41.04 ± 0.1^{c}	39.38 ± 0.2^{d}	45.36 ± 0.02^{a}	43.74 ± 0.2^{b}
	Polyunsaturated fatty acids (PUFA)					
C18:2,cis	9.26 ± 0.15	10.32 ± 0.32	10.5 ± 0.09	10.3 ± 0.02	11.08 ± 0.12	10.6 ± 0.17
C18:3 cis	0.22 ± 0.02^{c}	0.39 ± 0.02^{a}	0.28 ± 0.03^{b}	0.39 ± 0.02^{a}	0.24 ± 0.17^{c}	0.31 ± 0.01^{b}
C20:2 cis	0.02 ± 0.04	ND	ND	ND	ND	ND
C20:5 cis	ND	ND	ND	ND	ND	0.13 ± 0.0
Total PUFA	9.50 ± 0.02^{c}	10.7 ± 0.01^{b}	10.3 ± 0.03^{b}	10.4 ± 0.1^{b}	11.3 ± 0.08^{a}	11.04 ± 0.04^{a}
	Trans fatty acid (TFA)					
C18:2, trans	0.17 ± 0.01	ND	0.05 ± 0.06	ND	ND	0.10 ± 0.07
Total TFA	0.17 ± 0.01^{a}	ND	0.05 ± 0.06^{c}	ND	ND	0.10 ± 0.07^{b}
P/S ratio*	0.21	0.21	0.22	0.22	0.26	0.26
n6/n3 ratio**	42	26.5	37.5	26.4	46	24.1

Mean ± standard deviation of replicate analysis (n= 5)
Values with the same superscripts within the column are not significantly different (p < 0.001)
*P / S ratio = ratio of poly-unsaturated to saturated fatty acids, the standard value is ≥ 0.4
**n-3 / n-6 ratio = ratio of ѡ-3 and ѡ -6 fatty acids

The chemical characteristics of the extracted oil from food samples are presented in Table 2. The free fatty acid, acid value, expressed as percentage oleic acid, and peroxide value were highest in fried rice, while iodine value was highest in hot dog.

The concentration of pro-vitamin A carotenoids and tocopherol is presented in Table 3. The result indicated that α-carotene and β-carotene were highest in jollof rice and cryptoxanthin in fried rice. Thus, retinol activity equivalent (RAE) was highest in jollof rice, followed by fried rice and lowest in meat pie. The tocopherol content (mg α-Tocopherol / 100 g) was highest again in jollof rice and lowest in beef roll.

Table 2. Chemical Properties of oil extracted from Fast Foods

Samples	% Free fatty acid	Acid value (as oleic acid)	Iodine value(mg/100g))	Peroxide value (meq / kg)*
Jollof rice	3.6 ± 0.1[c]	7.29 ± 0.2[c]	26.6 ± 0.01[c]	2.26 ± 0.02[d]
Fried rice	6.5 ± 0.3[a]	12.9 ± 0.4[a]	63.5 ± 0.1[b]	3.28 ± 0.01[b]
Meat pie	4.5 ± 0.22[b]	8.97 ± 0.3[b]	63.4 ± 0.08[b]	4.62 ± 0.04[a]
Beef roll	2.7 ± 0.12[d]	5.33 ± 0.03[e]	26.6 ± 0.02[c]	2.25 ± 0.2[d]
Doughnut	2.1 ± 0.08[e]	4.20 ± 0.1[f]	25.4 ± 0.001[c]	1.80 ± 0.06[e]
Hot dog	3.2 ± 0.12[c]	6.17 ±0.2[d]	76.0 ± 0.04[a]	2.87 ± 0.02[c]

Mean ± standard deviation of replicate analysis (n=5)
Values with the same superscripts within the column are not significantly different (p < 0.001)
*meq/kg is milliequivalent peroxide per kg sample

Table 3. Pro-vitamin A Carotenoids, Tocopherol, Retinol Activity Equivalents and Vitamin E Activity of Fast Foods

Sample	α-carotene	β-carotene	Cryptoxanthin	Tocopherol	RAE*	VIT E**
Unit / 100 g		µg		mg α-T	I.U.	
Jollof rice	195 ± 0.2[a]	398 ± 12[a]	44.6 ± 0.4[b]	25 ± 1.0[a]	86.3 ± 0.4[a]	58
Fried rice	70 ± 3.5[b]	63 ± 3.0[b]	61 ± 0.9[a]	22 ± 0.4[b]	21.4 ± 1.0[b]	49
Meat pie	10.9 ± 0.3[e]	16.4 ± 0.5[c]	12 ± 0.6[e]	15 ± 0.3[c]	4.6 ± 0.4[d]	33
Beef roll	24.6 ± 0.7[d]	28.3 ± 3.0[c]	19.3 ± 0.5[d]	10 ± 0.07[d]	8.36 ± 1.2[c]	22
Doughnut	35.5 ± 1.0[c]	18.9 ± 0.8[c]	41 ± 2.0[c]	22 ± 0.6[b]	9.45 ± 0.7[c]	48
Hot dog	39.2 ± 0.5[c]	26.6 ± 0.7[c]	10.5 ± 3.0[e]	15 ± 0.5[c]	8.50 ± 0.5[c]	33

Mean ± Standard deviation of replicate analysis (n=5)
Values with the same superscripts within the column are not significantly different (p < 0.001)
*RAE – Retinol Activity Equivalent
**VIT E- Vitamin E Activity

5. Discussion

5.1. Fatty Acids

Palmitic acid, the predominant saturated fatty acid, as well as lauric and myristic acids which were found in smaller concentrations in the fast food samples investigated, have been implicated in the aetiology of heart diseases by raising plasma low density lipoprotein cholesterol. Stearic acid however has been shown not to affect plasma cholesterol concentrations [12].

The fast foods analyzed contained oleic acid as the major unsaturated fatty acid while linoleic acid (C18:2 n-6) accounted for 90% of the PUFA and α-linolenic acid was less than 1.0%. The ratio of n-6 to n-3 fatty acid ranged from 24:1 to 46:1. The beneficial effects of PUFAs depend on the ratio of the omega-6 (n-6) fatty acids to omega-3 (n-3) fatty acids.

It is generally accepted that the ideal proportion of n-6 to n-3 fatty acids is about 4:1, while the World Health Organization recommended a range of 10:1 to 5:1 [13]. The ratios observed in this study for meat pie, beef roll and fried rice were within the range of 20:1 to 30:1 reported in Western diets, which have been suggested to favour a pro-thrombotic and pro-aggregatory state [14], while the other samples had higher ratios. Linoleic acid and α-linolenic acid are important essential fatty acids, the body metabolizes both into arachidonic acid and docosahexaenoic acid, respectively. They could also be valuable dietary supplements for the *de novo* synthesis of icosanoids, whose function is to militate against dysfunction of cardiovascular, renal, reproductive, gastro-intestinal and immune systems [12]. The ratio of poly unsaturated to saturated fatty acid (P / S) ranged between 0.21 and 0.26; this value is lower than 0.4 or more recommended for a healthy life.

The content of lino-eladic acid (C18:2 trans), a major trans isomer found in the food samples, was significantly (p < 0.05) higher in doughnut (0.17%) than that of fried rice (0.10%) and hotdog (0.05%). The percentage trans fatty acid reported in this work was lower than a range of 0.8 to 3.6% reported for Iranian fast foods [15]. The presence of trans fatty acid in some fast foods could be of health concern, because trans fatty intake is considered to be hyper-cholesteromic.

Eicosapentaenoic acid (EPA) (C20:5) was reported only in fried rice while docosahexaenoic acid (DHA) (C22:6) was absent. The benefits of these long chain poly unsaturated fatty acids have been well documented to include anti-atherogenic, anti-thrombotic and anti-inflammatory effects and overall increased intake leads to a reduced risk of coronary health diseases [3].

The quality assessment of the foods indicated that the free fatty acid content ranged from 2.1 to 6.5%. A value ranging from 0.61 to 1.29% has been reported for traditional and factory processed anhydrous butter fat (samn) samples. Though, the ideal level of free fatty acid in processed vegetable oil is 0.5% [16]. Free fatty acid has been reported to play a very important role in the aroma and flavour, and also contributes to the organoleptic quality of foods when present in adequate concentration. Free fatty acid content is an index of lipase activity and an indicator of freshness, storage time and stability of many fat rich foods. It is well known that free fatty acids are more susceptible to lipid oxidation, leading to reduced smoke point, rancidity and production of off-odour compared to intact fatty acids in the triglycerides [17].

Acid value, which is used as an indicator for edibility of oil ranged from 4.20 to 12.9. The acid value of 14.04 has been reported for palm kernel oil, whereas, Pearson [18] reported acid value of 4 for soybean and sunflower oil. The iodine values (25.4 to 76.0 mg /100 g) obtained in this study indicated that the oils contain appreciable level of unsaturated bonds. Adequate methods to protect the food from deterioration should be put in place in order to prolong its shelf life. Peroxide value ranged from 1.80 to 4.62 meq / kg. The peroxide value between 1.5 and 2.5 have been reported for anhydrous butter fat (samn) prepared by traditional and industrial methods [16]. The

peroxide value obtained in this study fell within the acceptable limit of 8.0 meq / kg for human consumption, but secondary oxidation products, which were not analyzed, could influence the health of consumers if present.

5.2. Lipophilic Vitamins

This study showed that jollof rice contained 195, 398 and 45 ug / 100g, of α-carotene, β- carotene & cryptoxanthin, respectively, whereas meat pie recorded the least value. The values obtained in this study were within the range of 14 to 368 µg / 100 g reported for snack foods and 114 – 136 µg / g, 63 – 584 µg / g recorded for leafy vegetables and fruits, respectively [19, 20]. From the study, jollof rice and fried rice, whose preparation involved addition of ingredients such as red pepper, tomato and vegetable oil, which are veritable sources of carotenoids, recorded a higher content of carotenoids than fried and baked fast foods.

The retinol equivalent (4.6 to 86.3 µg / 100 g) was lower than the 246 to 627 µg / 100 g recorded for ready-to-eat sweet potato [21]. The low carotenoid content of fast foods may contribute significantly to vitamin A deficiency in Nigeria, unless the meals are balanced with preformed vitamin A sources, which are not easily accessible and affordable [22].

The values obtained for tocopherol and vitamin E activity ranged from 10 to 25 mg / 100 g and 22 to 58 mg / 100 g, respectively. These values were within the range of 30.6 to 34.6 mg/100g reported for raw and cooked fat trim obtained from beef [23]. The recommended daily allowance for vitamin E is 15 mg α-tocopherol or 33 I.U. However, the recommended allowance for vitamin E varies according to total lipids [4]. It has been suggested that different individual compounds, with variable anti-oxidant activities may provide additional protection against oxidative stress when ingested simultaneously. A combination of lipophilic antioxidants resulted in an inhibition of lipid peroxidation significantly greater than the sum of the individual effects [24]. This suggests that a combination of carotenoid and tocopherol in adequate proportion might provide dietary supplement to fight diseases associated with oxidative stress.

6. Conclusion

The findings of this study indicated that fast foods are characterized by a high level of saturated fatty acids. The ratio of n-3 to n-6 fatty acids did not conform to that recommended for a healthy diet. The trans fatty acid was found to be present, the content of carotenoids were low while tocopherol content could adequately provide the requirement for vitamin E. All the factors enumerated could predispose regular consumers of fast food products to cardiovascular and coronary heart diseases.

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