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Full Length Research Paper

The quality and infrared determination of trans-fatty acid contents in some edible vegetable oils

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The analysis of some edible vegetable oils available in the Cape Coast metropolis in Ghana has been carried out by physical and chemical methods quality determination and comparative analysis. The parameters investigated were the specific gravity, refractive index, iodine value, saponification value, acid value, free fatty acid content, percentage impurity. The specific gravities of the oils ranged from 0.9096 to 0.9984 whilst the refractive indexes were from 1.445 to 1.507. The saponification value ranged from 187 for 'Werewere to 244 for coconut while the iodine values varied from 9.5 for coconut to 135 for safflower. The other parameters were from 0.187 for soyabean oil to 6.89 for 'Werewere oil for the acid values and between 0 to 6% for the free fatty acid of the oil samples. The trans- fatty acid contents of the oils were also investigated by Fourier Transform Infrared (FTIR) spectroscopic method by the determination of the transmittance ratios of the absorbances at 966 cm^{-1} in the infrared spectra of the oils. The trans-fatty acid contents ranged from minimum of 0% for olive oil to a maximum of 6% in Werewere oil. The results indicated that the oils were of good quality and that the amounts of trans-fatty acid were not appreciably high to pose any health risk to consumers.

Keywords: Vegetable oil, physicochemical characteristics, infrared spectroscopy, trans fatty acid content.S

INTRODUCTION

Vegetable oils contain fats. These are triglycerides and contain fatty acid chains. In the body, triglycerides are metabolized to fatty acids by enzyme called lipases. During the manufacture of oils in the food industry, cis-trans isomerisatiom may occur in unsaturated fatty acid chains which results in increased concentrations of the (E)-isomer of an unsaturated fatty acid. The lipid content in food has always been of interest to health-conscious consumers. These acids have been implicated in a variety of conditions, including heart disease, cancer, stroke and diabetes (Housecroft and Constable, 2010). Ingestion of trans-fatty acids (TFA) appears to increase blood cholesterol, in particular the ratio of low-density lipoproteins (LDL, or 'bad" cholesterol) to high-density lipoproteins (HDL, "good cholesterol). TFA's appear to exhibit harmful effects on the heart that are similar to those shown by saturated fatty acids (Pavia et al., 1998). The adverse effect of TFA's on the ratio of total cholesterol to high-density lipoprotein cholesterol is twice that of saturated fatty acids. In response to such findings and public interest the U.S. Food and Drug Administration (FDA) required trans-fat content labeling for all foods beginning January 2006 (Walker et al., 2007).

By using a characteristic infrared spectroscopic absorption arising from an (E)-HC=CH vibrational mode which occurs at 966 cm^{-1}, it is possible to measure the amount of (E)-isomer in a sample of fat and oil (Housecroft and Constable, 2010). This would enable the health risks associated with fat and oil in diets to be estimated.

A variety of analytical methods to measure trans-fats have been utilized over the years. Gas chromatography of fatty acid methyl esters (FAME's) generated from transesterification of triglycerides has been one of the most popular methods. Recently FTIR attenuated total reflectance (FTIR-ATR) fitted with Nicolet OMNIC

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Table 1. Oil samples investigated in the present study.

LOCALLY PRODUCED OIL		IMPORTED/COMMERCIALLY PRODUCED OIL			
No	**NAME**	**No**	**NAME**	**Type**	**Manufacturer**
1	"Egusi'oil (EGO)	5	Borges oil	Olive	Aceites Borges, Spain
2	Werewere'oil(WSO)	6	Filma oil	Palm	PT Smart, Indonesia
3	Coconut oil (CSO)	7	Zade oil	Safflower	Helvacizade, Konya, Turkey
4	Groundnut oil (GSO)	8	Unoli oil	Soybean	Elburg Global BV, Holland
		9	Frytol oil	Palm oil	Unilever, Ghana

software in absorbance mode has been employed (Walker, 2007).

We describe the quality of cooking oils available in open-air market in Cape Coast metropolis in Ghana implementing the alternative analytical method of infrared spectroscopy and transmittance ratio for the determination of trans-fat in these oils. The main aim of this study was to evaluate the quality and triglycerides content in oils available in the Cape Coast metropolis and to ascertain the risk they pose to consumers. Concerns over the health and nutrition of the public, particularly over the average fat intake of oil consumers can be addressed through oil quality analyses. As the average Ghanaian's appetite for snack foods continues to grow along with health problems arising from obesity a demand for satisfying foods that are less fattening will be strong. The production of cooking oils with such property could curtail the public's appetite for fatty foods leading to an increased intake of fruits, vegetables and other healthy foods.

MATERIALS AND METHODS

The 9 oil samples investigated and shown in Table 1 were obtained in January 2012. The locally produced oils were purchased from the "Kotokuraba" Market in Cape Coast while the commercially produced/imported oils were purchased from the "Sonturk" Supermarlet in Cape Coast. The locally produced oils from "Werewere", (*Cucumeropsis mannii,* WSO) and "Egusi' (*Citrillus colocynthis,* ESO) plants were extracted from their seeds obtained from the market.

General Lipid Extraction Procedure of ESO and WSO

About 400 grams of powdered seeds were extracted with 1L petroleum ether (40-60°C) in a Soxhlet apparatus on a water bath for 8 hrs. The extract was concentrated to dryness under pressure at 40^0C to dryness. The pale yellow oil obtained was weighed and stored in a sealed dark bottle under nitrogen until analysis.

General Experimental procedure

Specific gravity was determined by using a specific gravity bottle. Refractive index was measured with an Abbe refractometer (Atago Co. Ltd, Tokyo, Japan) equipped with a thermostat circulator. Iodine values (Wij's solution method) were determined by using AOCS official method Ka-9-51, 1959 Nov, The saponification values were determined by using AOCS official method L-7a-57, 1959. Acid value (AV) and free fatty acid (FFA) contents were determined by titration method using AOCS official method Ca-5a-40, 1959, Nov. and Cd 3d-63. The percentage impurity was by the Whatman filter paper method (Cocks and Rede van, 1966). All experiments were run at least twice and the mean values were reported. Infrared spectra were taken on Shimatzu 8201A Series FTIR spectrophotometer in the Chemistry Department of the University of Cape Coast, Ghana and the oils were examined as neat samples.

RESULTS AND DISCUSSION

Fatty Acid Composition of Oils

All the oil samples were pale yellow in colour. The fatty acid compositions of the oils according to their types are well known (Pavia et al., 1998). Coconut oil (CSO) is composed of mainly of lauric acid which accounts for 41-50%. Groundnut (GSO) has 50-70% oleic acid. About 69-84% of olive oil is composed of oleic acid and "Unoli" oil which is made from soyabean oil has 50-59% linoleic acid. "Frytol" oil from palm oil has 38-40% oleic oil whiles 70% of "Zade"oil prepared from sunflower is composed mainly of linoleic oil. "Filma" (palm oil) has 60% linoleic acid. "Werewere" (WSO) is composed of four fatty acids: palmitic, stearic, oleic, and linoleic acids. Linoleic (C18:2) and oleic (C18:1) acids are the two most prominent fatty acids at 58.8% and 15.5%, respectively (Opoku-Boahen et a.l, 2012). The "Egusi" oil (ESO) has been determined to contain palmitic acid (10.48%), stearic acid(9.72%) oleic acid (17.95%) and linoleic acid (61.41%) (Solomon Giwa et al., 2010). The predominant fatty acids in all the oils are therefore palmitic, stearic, oleic and linoleic acids.

Studies have however indicated that fatty acid comp-

Table 2. Physicochemical properties of oil samples

Parameters	CSO Coconut oil	WSO Werewere' oil	GSO Groundnut oil	Olive oil	Unoli (Soya-bean oil	Frytol (Palm oil)	Zade (Sunflower)	ESO (Egusi)	FOM (Palm Oil)
Lipid content (%)	ND	37.15	ND	ND	ND	ND	ND	37.13	ND
Specific gravity	0.9109	0.9158	0.9096	0.9121	0.9163	0.9153	0.9912	0.9228	0.9984
Refractive index(30^{o}C)	1.445	1.466	1.463	1.466	1.463	1.507	1.471	1.469	1.465
Iodine value	9.518	128.49	75.51	88.838	135.149	57.105	120.555	107.865	54.181
Saponification value (mgKOH/g)	244.04	187.23	201.96	241.23	190.74	238.45	235.62	218.79	213.18
Acid value *(mgKOH/g)**	5.236	9.537	3.82	0.374	0.187	0.561	0.314	3.07	0.449
Free fatty acid(%) *****	4.50	3.44	0.24	4.33	4.28	4.33	5.04	0.49	6.04
Percentage impurity (%)	3.50	3.68	5.5	3.20	3.23	3.24	5.0	7.0	4.5

osition of some Cucurbitaceae oils in Cameroon including *C. manii* (WSO) can be affected by region of cultivation (Achu et al., 2005). Moreover composition of fatty acids varies depending on several factors including variety, growing area, climate and ripeness (Gohari et al., 2011).

Physicochemical properties of the oil samples

Physical properties of lipids derive directly from their chemical structures and functional groups and directly influence the functions of lipids in foods and the methods required for their manipulation and processing. They can also be used to assess the purity or quality of lipid material in reference to known standards or preferred characteristics (Nichols and Sanderson, 2003). The results of the physico-chemical analyses of the oils are represented in Table 2. The oils from the seeds of 'Werewere' (*Cucumeropsis mannii,*WSO) and "Egusi' (*Citrillus colocynthis,* ESO) were obtained by extraction with petroleum ether. The "Werewere" seed oil (WSO) and 'Egusi' seed oil (ESO) obtained were 37.15% and 40% respectively. These were slightly lower than in other studies in which yields of 52% has been reported for *Citrullus colocynthis* (Badifu, 1993). However Sawaya (1983) and Singh and Yidawa (1978) reported oil yield of 24% for *Citrullus colocynthis* whole seeds and mean range of 24.56 to 34.38% for seeds of *Citrullus* species. Whereas the seeds used in this experiment were unroasted, the seeds analyzed in other studies were generally roasted before extraction of the oil. Roasting has been shown to yield more oil, probably by reducing the moisture content of the kernel and allowing for easier rupture of the cell walls. Badifu, (1993) also noted that heating denatures protein, leading to the release of more oil from the kernel. It has also been claimed that differences in oil content can be attributed to genetic and climate conditions (Stevension et al., 2007). The contents of the seeds in the present study were found to exceed or comparable to that of some common edible oils such as cottonseed (22-24%), safflower (30-35%, soybean (18-22%), rapseed (40-48%) and olive (12-50%) (Nichols and Sanderson, 2003). Therefore the seeds of 'Werewere' (*Cucumeropsis mannii,*WSO) and "Egusi' (*Citrillus colocynthis,* ESO) can be considered as potential sources of vegetable oil for domestic and industrial purposes.

The specific gravities (SG) of the oils ranged from 0.9096 for groundnut oil (GSO) to 0.9912 for "Zade' (safflower) oil. Each of the oils specific gravity was well aligned with the following SG ranges described by previous studies : Coconut 0.9259, peanut(arachis) 0.917-0.9209, corn (maize) 0.9213-0.9250, cotton seed 0.922-0.925, olive 0.9150-0.9180, palm 0.9210-0.9240, palm kernel 0.9119, rapeseed 0.9133-0.9168, safflower 0.9246-0.9280, sesame 0.9203-0.9237, soja beans 0.924-0.9279, and sunflower, 0.924-0.9258 (Subrahmanyam, 1994).

Both iodine value and refractive index are important characteristics which determine the degree of saturation or unsaturation of fats and oils. The refractive index is used by most processors to measure the change in unsaturation as the fat or oil is hydrogenated. The refractive index of oils depends on their molecular weight, fatty acid chain length, degree of unsaturation and degree of conjugation (Nichols and Sanderson, 2003).

The refractive index of the oils under investigation ranged from 1.445 for coconut oil (CSO) to 1.507 for Frytol (palm oil). These values were within the range reported by Lazos (1986) for vegetable seed oils. Pure oils have marked ranges of refractive index and density; thus, the degree of variation of a typical oil from its true values may indicate its relative purity (Gohari et al., 2011). The oils investigated were therefore of pure quality.

The iodine value (I.V, g of I_2/100g) of oil is a measure of the unsaturation of fat or oil. Saturated fats or oils have low iodine values and unsaturated fats or oils have high iodine values. Usually fats and oils with high iodine values make softer soaps and fats and oils with low iodine values make harder soaps. The iodine values ranged from a low value of 9.5 for coconut oil (CSO) to a high value of 135 for "Unoli" (soyabean) oil. The iodine values for all the oils were consistent with the values in the literature (Pocklington, 1990) indicative of edible oils with good quality. The relatively low iodine values (less than 100) for coconut oil (9.5), groundnut oil (75.5) and palm oil (54-57) indicate a high degree of saturation classifying them as non-drying and hence unsuitable for the production of paints, vanishes and surface coating (Nagre et al., 2011). They are however good sources of edible oils.

Saponification value S,V. is an indicator of the average molecular weight and hence chain length. It is inversely proportional to the molecular weight of the lipid (Gohari et al., 2011). High saponification value (250mgKOH/g) of fats and oils are due to the predominantly high proportion of shorter carbon chain lengths of fatty acids (Kirk and Sawyer, 1991). Low molecular weight (short to medium chain) fatty acids have more glyceride molecules per gram of fat than high molecular weight acids. Each glyceride molecule requires three KOH molecules for saponification, hence the more the glyceride molecules the greater the saponification value (Aurand et al., 1987 as cited in Nagre et al., 2011). The saponification values ranged from 187 for "Werewere (WSO) to 244 for Coconut oil (CSO) and were in agreement with those reported in the literature indicating that the fatty acids in the oils have almost the same number of carbon atoms in them. This is because the four fatty acids present in significant quantities in all the oils are palmitic, stearic , oleic and linoleic acids which are all basically medium chain fatty acids and this accounts for the high saponification values of the oils samples (Nagre et al 2011).

The Acid value (A.V) and the percentage Free fatty acid (calculated as oleic acid, % FFA) of the oils were very low. The maximum acid value (AV) was 9 (mgKOH/g) for "Werewere (WSO) oil while the maximum free fatty acid (%FFA) was 6% for Filma (palm oil). The low values for %FFA and acid value could be attributed to the removal of the inherent free non -fatty resin acids as well as the free fatty acids generated during extraction or storage of the oils. Acid value gives an indication of the suitability of the oil for direct consumption. The Codex Alimentarius Commission expressed the permitted maximum acid values of 10 and 4 mg KOH/g for virgin palm and coconut oils respectively (Alfawaz, 2004 as cited in Gohari et al., 2011). It can be concluded that "Werewere oils (WSO) might not be suitable for direct consumption since its acid value is greater than the maximum permissible acid levels of 4mgKOH/g fat or oil required for virgin fats and oils(Codex Alimentarius, 1992, as cited in Nagre et al., 2011).

Crude vegetable oils contain impurities that are required to be removed to improve the quality as well as maintaining the nutritional values, making it useable as well as "edible" and safe for human consumption. These impurities are chlorophyll, non-glycerides and non fatty acid substances, suspended particles, metal traces as well as some coloring pigments and waxes (Cocks and Rede van, 1966). The percentage impurities in the vegetable oil ranged from the lowest value of 3.23 for olive oil to the maximum value of 7.0 for "Egusi" (ESO) oil. Generally the commercially produced oils investigated had lower impurities than the locally produced oils indicating that the locally produced oils were not refined.

FTIR analysis for TFA contents in oil samples

Theory

The baseline method for quantitative FTIR analysis involves the selection of an absorption band that is separated from the bands of other matrix components. A straight line tangent to the absorption band enables the values of P_o and P to be obtained as shown in Figure 1.

The value of the absorbance is given by the expression log (P_o/P). The value of the absorbance when plotted against the concentrations for a series of standard solutions enables the unknown concentration to be determined from this calibration curve. The use of such ratios eliminates many possible errors, such as changes in instrument sensitivity, source intensity and adjustment of the optical system (Patnaik, 2004). The calculated values for the absorbances (log(P_o/P) for various oil samples at 966 cm^{-1} are shown in Table 3.

In a similar experiment the area under the absorption peak at 966 cm^{-1} in the infrared spectra for various edible oils and fats were obtained as shown in Table 4

This information enabled the transmittance (log P_o/P) to be converted to appropriate area under the absorption peak at 966 cm^{-1} relative to olive oil which has a value of 1.1. In the same experiment the areas under the absorption peak at 966 cm^{-1} and the contents of (E)-triglycerides (%) by weight in sample were reported (Table 5). The calibration curve determined from the information is also shown (Figure 2).

The amount of (E) –triglycerides in the oil samples under investigation derived from the calibration curve are

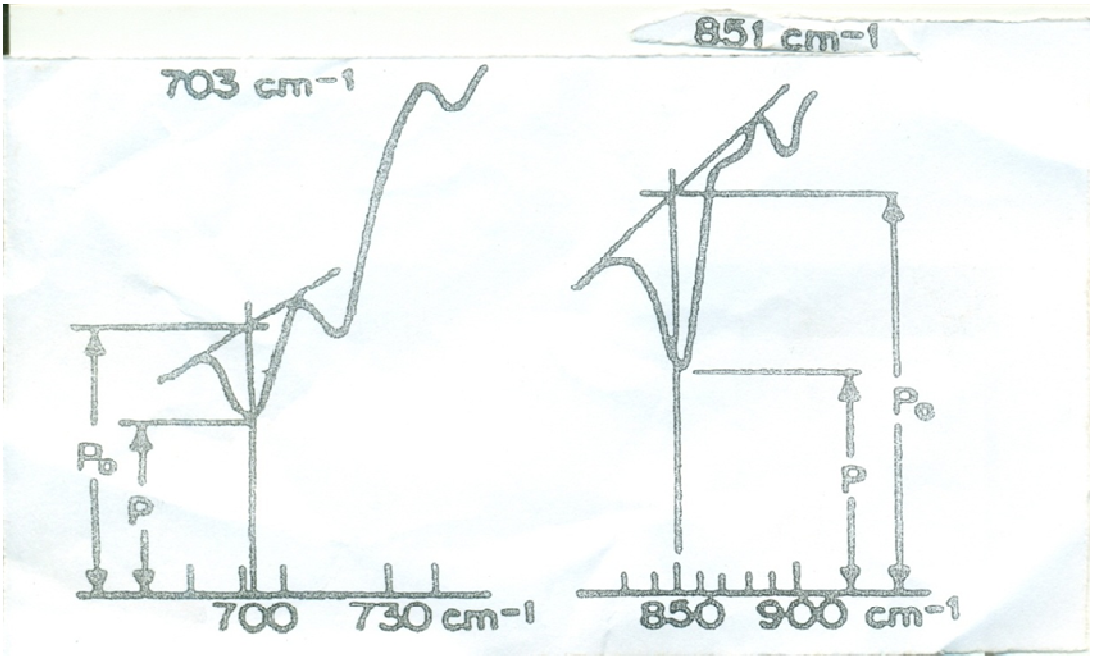

Figure 1. Baseline method for calculation of the transmittance ratio (Adapted from Patnaik 2004).

Table 3. Calculated absorbances of Vegetable oil samples.

Vegetable Oil Sample	Log (P_o/P)	Equivalent values for the area under the absorption peak at 966 cm^{-1}. [Log (P_o/P) x1.1/0.04493]
Coconut (CSO)	0.05614	1.37
Werewere (WSO)	0.08955	2.19
Groundnut oil (GSO)	0.04336	1.06
Olive oil	0.04493	1.1
Unoli(soybean oil)	0.04921	1.20
Frytol (Palm oil)	0.07463	1.83
Zade(Sunflower oil)	0.05115	1.14
Egusi (ESO)	0.06483	1.44
Filma (Palm oil)	0.05153	1.15

Table 4. Areas under the absorption peak for various oils (Adapted from Walker *et al* as cited by Housecroft and Constable, 2010).

Edible fat or oil	Area under the absorption peak at 966 cm^{-1}
Olive oil	1.1
Margarine	6.9
Corn oil	1.3
Oil for deep frying	4.7

Table 5. Absorption values of standard triglyceride samples (Adapted from Walker *et al.,* 2007 as cited in Housecroft and Constable, 2010).

Content of (E)-triglyceride(% by weight in sample)	0	10	30	50	100
Area under the absorption peak at 966 cm^{-1}	1.1	2.9	6.4	9.9	18.7

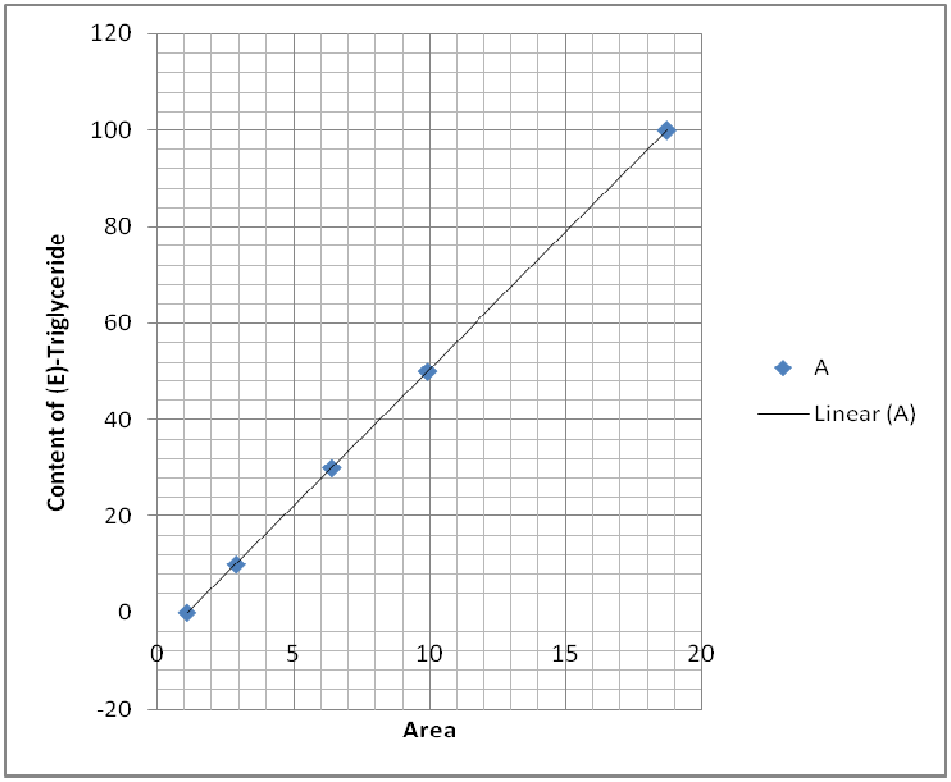

Figure 2. A plot of content of (E) triglycerides (% by weight in sample) against Area under the absorption peak at 966 cm^{-1} (y =5.6884x-6.369, r^2 = 1).

given as follows: Coconut (CSO) 2%, Werewere (WSO) 6 %, Groundnut oil (GSO) 0%, Olive oil 0%, Unoli (soybean oil) 2%, Frytol (Palm oil) 3.8%, Zade (Safflowerl) 1%, Egusi (ESO) 2% and Filma (Palm oil) 1%.

Trans fatty acids (TFA) are known to be ubiquitous in all food products. Dairy and beef fat typically contains around 3-6% TFAs (% of total fat) and levels in mutton and lamb can be somewhat higher. TFA levels in vegetable oils and liquid margarines are around 1%. Soft yellow fat spreads typically have between 1% and 17% TFAs, whilst harder stick margarines have higher levels. The TFA content of bakery products (rusks, crackers, pies, biscuits, wafers etc.) vary from below 1% up to 30% of total fatty acids. Some breakfast cereal with added fat, French fries, soup powders and some sweet and snack products have been shown to contain high TFA levels (20-40% of total fatty acids). However, surveys have shown that levels of TFAs appear to be decreasing in these products as manufacturers reformulate to remove hydrogenated oils if present.(Food Safety Authority of Ireland, 2009).

The TFAs present in the oils analysed were within the range found in most common food products. In the EU mean daily intakes of TFAs for 14 different countries (Ireland not included) range from 0.5-2.1% and 0.8%-1.9% of total energy intake amongst men and women respectively. The major contributors to TFA in the diets of people in these 14 countries were edible fats and ruminant fat with bakery products and French fries being additional contributing foods in some countries. This intake level appears to be decreasing. However, as yet, there is no official guidance on the consumption of TFAs in the diet, other than we should not increase consumption of TFAs above the current level. The World Health Organisation recommended that "Food manufacturers should reduce the levels of trans isomers of fatty acids arising from hydrogenation".(Food Safety Authority of Ireland, 2009).

It is noteworthy that though the method of analysis of TFA's is simple and appropriate the analytical method for the quantitative determination of trans fatty acids has limitations. In general, the trans double bonds in the fatty acid must be isolated. Oils with certain substituents along the fatty acid chains can also interfere with the measurement, e.g hydroxylated fatty acids such as in castor oils (Walker *et al* 2007) and caution is needed in the interpretation of the experimental data.

CONCLUSION

The 9 oil samples analysed were of good quality in terms of the parameters investigated. The trans fatty acid

measurements were aligned with the levels noted in these food products as described by previous studies. The levels were not very high to pose immediate health risk to consumers

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