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Effects of Chemical Purification on the Fatty acid Composition of *Cairca papaya* and *Citrus sinensis* seeds oils

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ABSTRACT
The oils of *Citrus sinensis*(CS) and *Carica papaya*(CP) seeds were extracted with soxhlet extractor apparatus using *n*-hexane as the solvent. The oils were concentrated by distillation and degummed with water and phosphoric acid, which was thereafter neutralized. Gas chromatography flame ionization detector (GC-FID) was used to determine fatty acid fraction (FAF). The prominent acids in the crude and refined oils of *Carica papaya* seed were oleic acid (74.5 %), (75.0 %), palmitic acid (12.1 %), (12.3 %), stearic acid (5.9 %), (5.6 %) and Linoleic acid (4.3 %), (4.1 %) respectively. The major fatty acids obtainable in *Citrus sinensis* sample were oleic acid (62.8 %), (63.4 %), palmitic acid (25.3 %), (24.3 %), stearic acid (7.2 %), (7.5 %) and Linoleic acid (3.4 %), (3.6 %) for crude and refined oils respectively. The statistical test results revealed that there was no significant difference in the values for the crude and refined oils. Thus, the purification processes (degumming and neutralization) did not affect the percentage fatty acid fractions of the oil but only reduced the phospholipid contents.
Keywords: Soxhlet extractor, degumming, GC-FID, phospholipid and Fatty acid fraction.

INTRODUCTION
It has been reported that the sources of commercial edible oils and fats include oilseeds, fruit pulps, animals and fish (O' Brien *et al*., 2000). Oilseeds are considered to be the major sources. The most widely used method to obtain the oil from oilseeds is pressing, followed by solid-liquid extraction. The main solvent used in the extraction is commercial hexane, which is a mixture of aliphatic and cyclic hydrocarbons. The extraction step results in an oil/solvent mixture with about 25–30% oil content (Savoire *et al.,* 2013). The solvent is subsequently removed by evaporation until the hexane content in the oil is lower than 1%. It has been reported earlier that besides the oil, the solvent used extract certain undesirable compounds such as phospholipids, free fatty acids (FFA), pigments, sterols, carbohydrates, proteins and their respective degradation products (Verleyen *et al*., 2002). These are substances that may impart an undesirable flavour and colour and shorten the shelf life of the oil (Pagliero *et al.,* 2004). Crude vegetable oils undergo complex refining processes to achieve the desired quality. The process has remained unchanged in recent decades even though it presents numerous drawbacks including high energy requirements, loss of neutral oil, the need for large amounts of water and chemicals, loss of nutrients and disposal of highly polluted effluents (Subramanian *et al*., 2001, 2001a). The presence of gum in oil imparts higher loss during refining and reduces storage life of oil (Amit *et al.,* 2013). The removal of phospholipids has been reported to be the first step in the refining process, in which water and dilute acid are added to the oil in order to convert phospholipids into hydratable gums, which are insoluble in oil. The gums are then separated from the oil by filtering, settling or centrifugal action (Pagliero *et al.,* 2001). The major disadvantages of these processes are considerable oil losses, large amounts of wastewater and high energy consumption (Ochoa *et al.,*2001).

Two types of phospholipids are present in fixed oil: hydratable (HPL) and nonhydratable (NHPL), and they are removed during degumming process. Most of the phospholipids in crude oils are hydratable and can be

removed by water degumming (Carelli *et al.,* 1997). Non-hydratable oils are not removeable by water since they cannot swell and form gels or precipitate from oil (Szydlowska-Czerniak, 2007). Therefore, NHPL requires more complex process at increased temperature with the use of phosphoric acid, citric acid or other degumming substances. Partial neutralization of acid is required to avoid migration of phosphatides back to the oil phase during degumming (Kovari, 2004). Membrane technique is one of the recent technologies for degumming vegetable oils (Ochoa *et al.,* 2001). The latest methods so far are soft and enzymatic degumming processes. For the soft process chelating agent like ethylenediaminetetraacetic acid (EDTA) in the presence of emulsifying agent is necessary. It has been reported that soft method reduced the gum level to as low as approximately 5 mg kg^{-1} (Jamil *et al*., 2000). However, the high cost of EDTA has militated against its use in industry (Choukri *et al.,* 2001). Two kinds of enzymes: Lecitase 10L (pancreatic phospholipase A_2) and Lecitase Novo (microbial lipase) have been found to be effective for degumming in the industry (Yang *et al.,* 2006; Bo *et al.,* 2006). The aim of this article was to study the effect of phosphoric acid on the percentage fatty acid fractions during purification (degumming and neutralization) of *Carica papaya* L. and *Citrus sinensis* seed oils.

MATERIALS AND METHODS

Sample collection

Mature papaya fruits (*Carica papaya* L.) and (*Citrus sinensis*) orange were purchased from a local market in January 2014. The fruits that were orange greenish in color and free from any defect and injury were selected. They were cut into two longitudinal halves and the seeds removed manually. Their seeds were sun-dried and the orange seeds were deshelled to remove the kernel. The kernels were sundried also to ensure proper drying. The seeds were grounded with Akira blender into fine powder and kept in air tight containers for future use.

Extraction of Oil

The extraction of the oils from the seeds was carried out in a Soxhlet apparatus using analytical grade hexane (*n*-hexane) as extracting solvent. At the completion of the extraction process, the oil was concentrated by simple distillation and residual solvent removed at 50°C. The oil was kept in bottles before use. Each batch extraction(5 g in 30 ml of *n*-hexane) lasted for 6 hours on the average (Bouanga-Kalou *et al*., 2011).

Degumming of Oil

The oil was first heated to a temperature of 60ºC, then 0.01 % phosphoric acid (w/w) was added to the oil. The mixture was then stirred for 30 minutes. Water (2 %) was added to the oil and heated to 70-80ºC for nearly 15 minutes. After centrifugation, the gums separated out from the oil which was drained leaving the oil in the separating funnel (Pagliero *et al.,* 2004).

Neutralization of Oil

Neutralization of the oil was done in a beaker (250 mL). It was stirred with a mechanical stirrer at 60°C and calculated amount (10 %, w/w, excess over the stoichiometric quantity) of alkali (as 15 % w/v, solution in H_2O) was added slowly. Stirring was continued for 1 h and centrifuged at 150-200 rpm in a Remi (Model: C24, Mumbai). The separated soap was removed and the oil was washed with hot distilled water untill the oil becomes completely free from soap after testing with phenolphthalein indicator.

Fatty acid methyl esters (FAMEs) preparation

The extracted fat content (50 mg) of the sample was saponified for 5 min at 95°C with 3.4 ml of 0.5 M KOH in dry methanol. The mixture was neutralized using 0.7 M HCl. Exactly 3 ml of 14 % baron trifluoride in methanol was added. The mixture was heated for 5 min at 90°C to achieve complete methylation. The fatty acid methyl esters were extracted from the mixture with redistilled *n*-hexane and the process was repeated thrice. The content was concentrated to 1 ml for gas chromatography analysis (Aremu *et al.,* 2006).

Gas Chromatography (GC) analysis

Exactly 1 µl was injected into Hewlett Packard (HP) 6890 GC powered with HP Chemstation Rev.AO 9.01 (1206) software, equipped with flame ionization detector (FID). The column was packed with HP innowax (cross – linked P.E.S); 30.0 m column length; 0.32 nm I.D; 0.50 µm film thickness. The column initial temperature was 60°C for 3 mins, later increased at the rate of 8°C/min to 140°C, and maintained at this temperature of 140°C for 5 min and then increased to 250°C at 10°C/ min and maintained constant for 10 min. Injector and detector temperatures were 230°C and 275°C respectively. The carrier gas, nitrogen was maintained at 30.0 psi, while hydrogen pressure was at 22 psi and compressor air pressure was also maintained at 28 psi. FAMES peaks were identified by comparison of their retention time with those of a standard mixture obtained from Sigma Chemical Company.

Statistical Method

All measurements were performed in triplicate. The statistical analysis was carried out with the program Statgraphics Plus, Version 1.4 for Windows (Manugistic, Rockville, USA). The significance of differences between mean values of all measurements were determined at the p = 0.05 (5%) level using a one way analysis of the variance and the t-test.

RESULTS AND DISCUSSION

Plates 1 and 2 are the pictures of the extracted oils (*Carica papaya* and *Citrus sinensis*) after concentration.Table 1 represents the physicochemical parameters of the crude extracts; the percentage yields are 30 % and 28 % respectively; it has been reported earlier that any oil bearing seeds that can produce up to 30 % oil are regarded as suitable for industrial application (Alabi *et al.,* 2013).

The saponification value of CSSO (194) makes it a good candidate for production of soap which is far higher than that of CPSO (79). CSSO is semi-drying oil according to the result because of its iodine value (108) while CPSO is non-drying with iodine value (30). The low free fatty acids content in CPSO is indicative of low enzymatic hydrolysis that makes it better than CSSO in developing off flavour during storage (Bouanga-Kalou *et al*., 2011). The refractive index reflects the degree of unsaturation and chain length. Values obtained here (1.46 for CSSO and 1.47 for CPSO) are expected of oils with low iodine value and the presence of Oleic acid fatty in the proportion observed (Tables 2). The unsaponifiable values are 1.5 and 1.3 respectively. This is an indication that the steroidal and related components are low in the oil (Bouanga-Kalou *et al*., 2011).

The fatty acid composition of the crude *Carica papaya* seed oil (CCPSO) and refined *Carica papaya* seed oil (RCPSO) are shown in Table 2. The result showed that the major fatty acid found in crude and refined oils were oleic (74.5 %), (75.0 %) followed by palmitic (12.1 %), (12.3 %), stearic (5.9 %),(5.6 %) and Linoleic (4.3 %), (4.1 %), respectively. The results are comparable with the findings of Bouanga-Kalou *et al.* (2011) where they reported the major saturated fatty acids in *Carica papaya* seed oil were palmitic (15.22 %) and stearic (4.39 %) acids and the main unsaturated fatty acids are oleic (76.38 %) and linoleic (4.02%). From these results, it is obvious that there was no noticeable difference between the values obtained for the crude and the refined oils. The major fatty acid obtained were oleic (62.8 %), (63.4 %) followed by palmitic (25.3 %), (24.3 %), stearic (7.2 %), (7.5 %) and linoleic (3.4 %), (3.6 %) for crude (CCSSO) and refined oil (RCSSO) respectively. The results obtained (Table 2) are closely related to what Syed *et al.,* (2012) reported. No noticeable difference between the values obtained for the crude and the refined oils.

Plate 1: Extracted *Carica papaya* seed oilPlate

2: Extracted *Citrus sinensis* seed oil

Table 1: Physicochemical Parameters of *Carica papaya* and *Citrus sinensis* seed oil

SN	Parameters	CSSO	CPSO
1	Percentage Yield (%)	30.64	28.1
2	Saponification value (mgKOH/g)	194.3	79.38
3	Iodine value (gI_2/g)	108	30.2
4	Acid value (mg KOH/g)	51.4	47.12
5	Refractive index (28 ^{O}C)	1.468	1.47
6	Peroxide value	0.3	48.6
7	Specific gravity (24 ^{O}C)	0.842	0.85
8	Free Fatty Acid (FFA) (%)	26.7	1.27
9	Unsaponifiable Matter (%)	1.5	1.37
10	Stability Oxidative (hours)	78.86	
11	Density(kg m^{-3})	730	885
12	Viscosity(m.Pa)	36.5	67.7

CSSO:*Citrus sinensis* seed oil; CPSO is *Carica papaya* seed oil

By observing the mean in Table 3, it can be seen that the values for Crude *Carica papaya* seed oil (CCPSO) is the same with that of refined (RCPSO) (9.090) and ditto to the Crude *Citrus sinensis* seed oil (CCSSO) and refined (RCSSO) (12.500). These seem to support our hypotheses that the CCPSO is not different from RCPSO, and that the CCSSO is also not different from RCPSO. i.e

$$H_0: CCPSO = RCPSO \; vs H_1: CCPSO \neq RCPSO$$

and

$$H_0: CCSSO = RCSSO \; vs H_1: CCSSO \neq RCSSO$$

However, to ascertain whether these results are significant or due to chance, the Paired Samples Tests (PST) was conducted.

Similarly, the Standard Deviation (SD) in Table 3 shows that the gap in the values of the parameters in RCPSO is slightly higher than that in the CCPSO. This is the same for RCSSO and CCSSO. The standard error means here is an estimate of the standard deviation of the sampling distribution of the mean. A small value of this standard error means that a similar mean should be expected if the test was carried out again, but a high value indicates a lot of disparity predicted in the means. The standard error is a useful figure as it is used in the computation of significance tests comparing means, such as the t test, as conducted here to check for difference or relationship between the two samples.

GC-FID absorption spectra of the samples

Table 2: Percentage fatty acids of crude and refined *Carica papaya*and *Citrus cinensis*Seeds Oil.

Retention Time (mins)	Common Name	Carbon-chain	CPSO (%)	RCPSO (%)	CCSSO (%)	RCSSO (%)
14.44	Myristic Acid	C14:0	0.389	0.368	-	-
16.044	Palmitic Acid	C16:0	12.183	12.373	25.375	24.361
16.677	Palmitoleic Acid	C16:1	0.448	0.424	0.06	0.062
18.06	Stearic Acid	C18:0	5.954	5.67	7.264	7.506
18.941	Oleic Acid	C18:1	74.555	75.014	62.866	63.459
19.526	Linoleic Acid	C18:2	4.366	4.157	3.484	3.618
20.658	Linolenic Acid	C18:3	0.474	0.448	0.376	0.393
21.911	Arachidic Acid	C20:0	0.654	0.618	0.518	0.542
23.972	Behenic Acid	C22:0	0.36	0.344	-	-
24.885	Erucic Acid	C22:1	0.269	0.255	0.055	0.057
25.623	Lignoceric Acid	C24:0	0.343	0.324	-	-

CCPSO is Crude *Carica papaya* seed oil, RCPSO is Refined *Carica papaya* seed oil
CCSSOis crude *Citrus sinensis* seed oil and RCSSO is refined *Citrus sinensis* seed oil

Table 3: Statistical differences in samples

		Mean	N	Std. Deviation	Std. Error Mean
Pair 1	**Crude** *Carica Papaya* seed oil	9.0904	11	22.0311	6.6426
	Refined *Carica Papaya* seed oil	9.0904	11	22.1851	6.689
Pair 2	**Crude** *Citrus sinensis* seed oil	12.5	8	22.0758	7.8049
	Refined *Citrus sinensis* seed oil	12.5	8	22.1696	7.8381

Table 4: Samples correlations

		N	Correlation	Sig.
Pair 1	Crude *Carica Papaya* seed oil & Refined *Carica Papaya* seed oil	11	1.00	0.00
Pair 2	Crude *Citrus sinensis* seed oil & Refined *Citrus sinensis* seed oil	8	1.00	0.00

It is evident from Table 3, that the pairs of samples are highly correlated as the significant value is very low, below 0.01 (1%), and the coefficient of correlation is 1.

Table 5:Test of significant difference in samples

		Paired Differences					t	df	Sig. (2-tailed)
		Mean	Std. Deviation	Std. Error Mean	95% Confidence Interval of the Difference				
					Lower	Upper			
Pair 1	Crude *Carica Papaya* seed oil - Refined *Carica Papaya* seed oil	0.000	0.1936	0.0583	-0.1301	0.1301	0.000	10	1.000
Pair 2	Crude Citrus sinensis seed oil - Refined Citrus sinensis seed oil	0.000	0.4562	0.1613	-0.3814	0.3814	0.000	7	1.000

Table 5 is to test for significant difference between the pair of samples, $t_{(10)} = 0.000$; $P < 0.05$. The difference between the mean of CCPSO and RCPSO and between CCSSO and RCSSO is equal to zero, since both crude and refined samples have equal means.

The standard deviations are relatively small values (0.1936) and (0.4562), indicating that the corresponding values in each of the set of data :CCPSO and RCPSO; CCSSO and RCSSO are not significantly different. The standard error mean estimates the standard deviation of all the differences between sample means for sample sizes n= 11 and n=8 when the null hypothesis is true. This indicates the difference in the means that should expected by chance if the null hypothesis is true (Perry, 2004). The mean difference is 0.000 in both cases, which is less than the standard error of the mean of 0.0583 and 0.1613 respectively, this suggest that the two pairs of samples are not significantly different. Our calculated t-values are the ratios of mean and standard deviations.

$$t = \frac{0.000}{0.0583} = 0.000$$

$$t = \frac{0.000}{0.161} = 0.000$$

The 95 % confidence interval of the difference indicates that we are 95% confident that the true difference in means will be between the upper and lower limits. The sample mean difference falls between these two values; (-0.1301 and 0.1301) and (-0.381 and 0.381) for *papaya* and *sinensis*. The statistical analysis to compare the values of fatty acids of crude and refined *Caricapapaya* and *Citrus sinensis* seed oils revealed that the crudes and refined results are not significantly different from one another as we fail to reject the null hypotheses base on the P-value (1.000) > 0.05. The smaller the P-value, the stronger the evidence against the null hypothesis and as our P-value here is higher, we do not reject H_0; meaning that our samples are equal.

CONCLUSION

This research work has further confirmed the results earlier presented on the percentage acid compositions of *Carica papaya* oil by other researchers. Also it has been able to confirm that acid degumming process does not alter the percentage fatty acid compositions of oils during purification. The experimental results were upheld by the statistical analyses presented.

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