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COMPARATIVE ANALYSIS OF THE LIPID CLASSES, FATTY ACID AND PHOSPHOLIPID PROFILES OF THREE SPECIES OF CITRUS FRUITS

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Abstract: The oils extracted from the seeds of three species of citrus fruits were analyzed for their lipid classes, fatty acid composition, phospholipid profile and physicochemical properties. The seed oils were extracted using Soxhlet extraction method and the contents were analyzed using gas chromatographic techniques. The seed oils were rich in neutral lipids with lime seed oil having the highest percentage of 89.7 % and tangerine seed oil the lowest percentage of 84.83 %. The seed oils also showed the presence of glycolipids with values ranging from 6.95 - 8.90 %. The oils have high amount of total saturated and polyunsaturated fatty acid with values ranging between 35.8 - 40.3 % and 41.7 - 48.8 % respectively. The total phospholipid content was between 462 - 565 mg/100 g with very high concentration of phosphatidylinositol and phosphatidylcholine detected in the oil samples. The oils had very high percentage oil yield and saponification value. The iodine value of the oilseeds ranged between 102.3 - 135.5 I_2 g/100 g with lime oil having the highest value. The results showed that the oils have high amount of essential fatty acids and phospholipids which are major components needed for the development and growth of some vital organs in the body system. The chemical and physical properties of the citrus oils also showed that the oils will have good application in various industries like food, soap, cosmetics and medicine.

Keywords: *extracted oil, glycolipids, grapefruits, lime, phosphatidylcholine, tangerine*

INTRODUCTION

Lipids are large and diverse groups of naturally occurring organic compounds. These lipids perform different functions in the system of living organism such as transportation of fat soluble nutrients (sterols, vitamin) within the body system; they form structural elements of cell and subcellular components of hormones; they are also precursor for prostaglandin synthesis. Lipids have been found to be involved in some biological activities, which help the body to run smoothly in many ways like: helping the brain to work well, joint mobilization, energy production, help to coat the cells with lubrication therefore providing protective barrier for delicate organs of the body, and also help in internal cellular communication [1].

Lipids are divided into sub groups depending on their functional characteristics but the general classes of lipids are neutral lipids, glycolipids and polar lipids [2]. Neutral lipids are uncharged lipids and this comprise majorly mono-, di- and triacylglycerols, cholesterol and cholestery esters [2] Glycolipids are lipids with a carbohydrate attached by a glycosidic bond while polar lipids have a polar group as the head group example is phospholipid. The role of glycolipid in cells is to serve as markers for cellular recognition and also to provide energy. They function mainly in cell to cell interactions and thus help in cell recognition, regulation, growth, and apoptosis [3]. Phospholipids are derivatives of triglycerides. They are composed of a glycerol molecule with two fatty acids while the third carbon contains a phosphate group. They are constituents of cell membrane which regulate permeability; they also act as detergents to emulsify transportation of fat within the body. Phospholipids are components of human brain (20 - 25 %). They provide membrane for the fluidity of the brain, normal functioning and cognition of the brain [4].

The biological functions and health benefits of lipids have generated more interest in the profiling of lipids in vegetable oils. Grapefruit (*Citrus paradisi*), Lime (*C. aurantifolia*) and Tangerine (*C. reticulata*) are part of the numerous species of citrus group which are grown mainly for the production of juice and also as staple foods. The peels and seeds of these citrus groups which were usually regarded as waste have also been found to be good sources of essential oils which have been found to contain mixtures of compounds and also useful in many chemical industries [5]. Recently it has been shown that citrus seeds contain 24 - 56 % oil [6] which could contain different classes of lipids that have been found to be beneficial to health.

The profiling of fatty acid of oils extracted from different citrus seeds have been reported to contain essential fatty acids, monounsaturated fatty acids and triglycerides, [7 – 11] but there is paucity of information on the lipid classes and phospholipid profile of oils extracted from these citrus seeds. A critical look at the lipid profile of the oils from these citrus seeds will help to unveil the hidden nutritional, medicinal and industrial benefits of these seeds instead of regarding them as waste.

MATERIALS AND METHODS

Reagents

Diethyl ether, chloroform, hexane, dimethyl ether and sodium methoxide were purchased from Sigma Aldrich Chemie GmbH Germany, methanol and acetone HPLC grade were purchased from May and Baker Dagenham, England.

Collection of samples

The three fruits: grapefruit, lime and tangerine were obtained from farms in Ado Ekiti, Ekiti State, Nigeria. The seeds were removed, washed thoroughly and air dried before grinding.

Extraction of oil

The oils were extracted individually using Soxhlet extractor with diethyl ether for 8 hours at 50-60 ^{o}C after which the extracted oils were concentrated using rotary evaporator.

Determination of the lipid classes

600 mg of the extracted oil was fractioned into the major lipids classes: neutral, glycolipids and phospholipids by silica gel column chromatography. Neutral lipids were eluted with chloroform, glycolipids with acetone and phospholipids with methanol. 1.0 mL fractions were collected per minute and elution was monitored using thin layer chromatography. The thin layer chromatography separation was conducted on silica gel 60 F_{254}layers (20 x 20 cm thickness; Merck). The chromatogram was developed in a nitrogen atmosphere in petroleum ether/diethylether/ acetic acid (80 : 20 : 1 v /v /v) twice and was viewed under UV light (254 nm). The extract was concentrated by stream of nitrogen and percentage of the fractions that is free of solvent was determined by gravimetric method.

Determination of the fatty acid composition

The fatty acid composition was determined according to Cocks and Van Rede [12] with slight modification. 0.5 g of oil extracted was mixed with 3 mL of dimethylether and together with 0.2 mL of sodium methoxide to form a colloidal solution. The solution was allowed to settle, and centrifuged at 2500 rpm for 10 min with a Leaidal centrifuge to precipitate into solid. The solid was filtered with filter paper and the filtrate was kept for gas chromatographic analysis. 1 μL of the filtrate was injected into gas chromatography instrument (HP 6890 Powered with HP chemstation Rev. A 09.01 [1206] software) equipped with a flame ionization detector. The conditions were as follows: stainless steel column 30 m x 0.25 mm x 0.25 µm packed with HPINNOWAX; column temperature was 250 ^{o}C; carrier gas N_2, 35 mL·min^{-1}; H_2, 30 mL·min^{-1}. Oven temperature program - Initial temperature at 50 ^{o}C, First Ramping at 10 ^{o}C/min for 20 min maintained for 4 min, Second Ramping at 15 ^{o}C/min for 4 min, maintained for 5 min. Individual fatty acids were identified by comparing their retention times with a certified fatty acid methyl esters (FAME). The relative percentage of each fatty acid was quantified as percentage of the total fatty acids.

Determination of the phospholipid profile

The phospholipid content of the extracted oil was determined using the method of Raheja *et al*. [13] with slight modification. 0.01 g of the extracted fat was added to the test tubes, stream of nitrogen was passed over the oil to completely remove the solvent. 0.40 mL of chloroform was added to the content of the tube and it was followed by the addition of 0.10 mL of the chromo-genic solution. Content of the tube was heated at temperature of 100 ^{o}C in a water bath for about 1 minute 20 seconds. The content was cooled and allowed to attain room temperature; 5 mL of hexane was added and the tube with its content shaken gently several times. The solvent and the aqueous layer were allowed to be separated. The hexane layer was recovered and concentrated to 1.0 mL for gas chromatographic analysis. The gas chromatography instrument (HP 6890 Powered with HP chemstation Rev. A 09.01 [1206] software) equipped with pulse flame photometric detector was used for the analysis. Conditions were the same as described for fatty acid composition above.

Determination of the physicochemical properties

Physicochemical properties of the oil: acid, iodine, saponification and refractive index values were determined using methods described by Association of Official Analytical Chemists [14].

Statistical analysis

The obtained results from the analytical determinations were subjected to analysis of variance, chi square and kruskal-Wallis test using GraphPad Prism Program version 5 for Windows and the level of significance was set at $P < 0.05$.

RESULTS AND DISCUSSION

Lipid classes of the extracted oils

Table 1 shows the lipid classes of the oils extracted from the citrus seeds. The oils have very high percentage of neutral lipid which range between 84.8 % in tangerine and 89.7 % in lime. The content of glycolipids in the seed oils ranged between 6.95 and 8.90 %. Phospholipid was present in low amount in the oils. Glycolipids play important role in human health; they have been found to perform a variety of functions in living organisms because of their diversified structures [15]. The role of glycolipids in various biological systems is currently attracting a lot of attention thus different attempts to isolate glycolipids from various natural sources. Glycolipids have been found to act as anti-cancer agents along with some other immunosuppressive effects [16]. It has also been found that glycolipid could be used in pharmaceutical and cosmetic industries due to its effect on type 1 diabetics, Alzheimer's disease and intracellular bacterial infections [16].

Table 1. *Lipid classes of the oils extracted from the citrus species*

Lipid classes	Grapefruit [%]	Lime [%]	Tangerine [%]
Neutral lipids	88.0[a]	89.6[a]	84.8[a]
Glycolipids	6.95[a]	7.10[a]	8.90[a]
Phospholipid	0.80[a]	0.91[a]	0.85[a]
Free lipids	4.20[a]	2.12[a]	5.32[a]

Values followed by the same letters in the row has no significant differences ($p < 0.05$)

They can also be used in various foods, pharmaceuticals, cosmetics and nutraceutical formulations. Glycolipids were used as synergists in a detergent formulation made for manual dish-washing this is because majority of naturally occurring surfactants (bio-surfactants) are glycolipids [15].

Although, the amount of glycolipid in these oils is not too high, the advantage of molecules of glycolipid is that they show very high activity even at a very low concentration; therefore, the glycolipid present in the oils would be of importance in the cosmetic and pharmaceutical industry. The oils consist majorly of neutral lipids; neutral lipid fraction of vegetable oils mainly consists of triacylglycerols, however small amounts of partial glycerides (Monoacylglyceride MAG and diacylglycerol DAG) are always present. Neutral lipids perform most functions of lipids; they serve as fuel reserve of the body, they protect delicate internal organs, serve as insulating materials and also give shape to the body. The result showed that the oils have very high percentage of neutral lipid, although there was no statistical difference between the lipid compositions of the oils.

Fatty acid composition

The result of the fatty acid composition showed that the citrus oils has high percentages of palmitic acid among the saturated fatty acids thus resulting in a total saturated fatty acid content ranging between 35.8 and 40.3 % (Table 2) with lime seed oil having the highest amount. The observed result is in agreement with results of other authors [7 – 11, 17 – 18].

The oils are also high in unsaturated fatty acid, principally oleic and linoleic fatty acid thus classifying these fruits as sources of omega 6 fatty acid. Both Grapefruit and Tangerine seeds oils had high amount of total monounsaturated fatty acid than lime seeds oil (although not statistically significant) while lime seeds oil had higher percentage of poly unsaturated fatty acid (PUFA) than the other seed oils (Table 2). PUFAs are essential fatty acids and mammals lack the ability to synthesize them in the body [19]. Therefore, they are obtained from plant sources. The averagely high amount of PUFAs in these citrus species (especially lime) showed that they could be sources of this essential fatty acid when consumed regularly.

***Table 2**. Fatty acid composition of the citrus group*

Fatty acid	Grapefruit [%]	Lime [%]	Tangerine [%]
C14.0 [myristic acid]	0.747[a]	2.53[b]	0.865[c]
C16.0 [palmitic acid]	30.17[a]	22.97[a]	27.49[a]
C16.1 [palmitoleic acid]	0.381[a]	0.513[a]	0.475[a]
C17.0 [margaric acid]	0.121[a]	0.162[a]	0.148[a]
C18.0 [stearic acid]	5.43[a]	9.64[a]	6.01[a]
C18.1 [oleic acid]	20.18[a]	10.28[a]	20.06[a]
C18.2 [linoleic acid]	37.47[a]	39.29[a]	39.88[a]
C18.3 [linolenic acid]	4.18[a]	9.44[a]	3.62[a]
C20.0 [arachidic acid]	0.794[a]	4.46[b]	0.786[c]
C20.4 [arachidonic acid]	0.064[a]	0.086[a]	0.079[a]
C22.0 [behenic acid]	0.289[a]	0.390[a]	0.359[a]
C22.1[erucic acid]	0.044[a]	0.059[a]	0.055[a]
C24.0 [lignoceric acid]	0.139[a]	0.188[a]	0.174[a]
TSFA	37.69[a]	40.33[a]	35.83[a]
TMUFA	20.60[a]	10.85[a]	20.59[a]
TPUFA	41.71[a]	48.81[a]	43.58[a]

TSFA- total saturated fatty acid, TMUFA- total monounsaturated fatty acid, TPUFA – total poly unsaturated fatty acid, values followed by the same letters in the row has no significant differences ($p<0.05$)

This also showed that the oils could have great industrial applications.

Phospholipid profile of the oils

The result of phospholipid profile showed that lime seeds oil had the highest amount of Phosphatidylcholine (PC) while tangerine seed oil had the highest amount of phosphatidylinositol (PI) (Figure 1). All the three seed oils had almost equal amount of phosphatidylinositol; thus indicating that all the seeds could be good sources of dietary phospholipid. As observed by other authors [20, 21], phosphatidylcholine is usually the most abundant phospholipid in animals and plants because it is a member of the lecithin group which is the key building block of membrane bilayers. It is a major component of surfactant and plays major roles in lung maturation and readiness for effective gaseous exchange in human new-borns [21]. Phosphatidylcholine have been shown to have a mild improvement on the memory and cognition of 32 elderly people [21]. The total phospholipid content of the seed oils are 462.9, 565.0 and 557.5 mg/100g for grapefruit, lime and tangerine seed oils respectively.

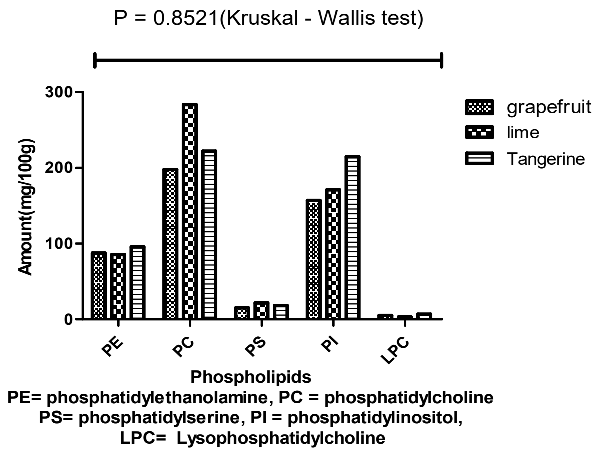

Figure 1*. Phospholipids profile of the citrus seeds oils*

The total amount of phospholipids in these fruits showed that these citrus fruits could serve as sources of phospholipid to the body system when consumed regularly. Thus, they could help the body system to get the health benefits of phospholipids most especially in the development of human brain. Phospholipid has been found to be useful in development of brain by providing the dynamic membrane with a suitable environment, fluidity and ion permeability that affects cognition of the brain positively. Thus addition of these fruits as drinks for growing infants could help in the development of their brain as they require rapid growth in the brain. The result of phospholipid profile observed in the present work is in agreement with the report of Adeyeye and Adesina [18] on the phospholipid profile of some citrus species.

Physicochemical characteristics of the citrus oils

The physicochemical characteristics of these oils as reported in Table 3 showed that these citrus groups could be good sources of oil; the oil yields ranged between 40 and 50 % with grapefruit having the highest and lime the lowest percentage. The oils also have high saponification and iodine values. The high saponification values of these oils coupled with their glycolipid content showed that they could be used as additives and flavors in washing liquid soap; this also justifies the use of lime as additives in liquid soaps. Lime oil has higher iodine value than grapefruit and tangerine oils, the higher iodine value of lime oil showed that it is a drying oil and thus could find application in paint and ink industry while grapefruit and Tangerine oils are semi drying oils. All the oils had very low peroxide value; this showed that the oils cannot easily go rancid [22]. The acid values of the oil fall with the Food and Agriculture Organization (FAO) codex standard for edible oils [23] thus confirming their edibility.

***Table 3**. Physicochemical parameters of the citrus oil*

Physicochemical parameters	Grapefruit	Lime	Tangerine
Kinematic viscosity [$mm^2 \cdot s^{-1}$]	38.20[a]	35.20[a]	36.50[a]
Saponification [$mg \cdot g^{-1}$ of oil)	186.80[a]	212.20[a]	195.40[a]
Peroxide [$meq \cdot kg^{-1}$]	0.85[a]	0.82[a]	0.84[a]
Iodine value [gI_2/100 g]	102.30[a]	135.50[a]	105.20[a]
Acid value [$mgKOH \cdot g^{-1}$]	1.15[a]	3.80[a]	1.20[a]
Specific gravity [$g \cdot cm^{-3}$]	0.79[a]	0.90[a]	0.77[a]
FFA [%]	2.00[a]	0.12[a]	3.14[a]
Oil yield [%]	50[a]	40[a]	48[a]

Values followed by the same letters in the row has no significant differences ($p < 0.05$)

This result is in agreement with the report of other authors on the physicochemical properties of some citrus seed oils [7, 10].

CONCLUSION

The present research has been able to throw more light into the lipid composition and the chemical characteristics of grapefruit, lime and tangerine seeds oils. It has also shown the presence of some minor components in the oils which could be of good nutritional values. Also highlighted are the proposed industrial applications of the oils, thus revealing the potentials of these underutilized seed oils.

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