IJAAR 5 (2017) 29-39 ISSN 2053-1265

International Journal of Advance Agricultural Research
www.bluepenjournals.org/ijaar

Physicochemical properties of refined and unrefined oils of gingerbread plum (*Neocarya macrophylla*) kernels from Guinea and Niger

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Article History
Received 24 December, 2016
Received in revised form 30 January, 2017
Accepted 02 February, 2017

Keywords:
Gingerbread plum kernel,
Unsaturated fatty acids,
Physicochemical properties.

Article Type:
Full Length Research Article

ABSTRACT

In this study, gingerbread plum kernel oil from Guinea (GPKOG) and Niger (GPKON) were refined (R-GPKOG and R-GPKON), studied and compared for their fatty acid compositions, phytosterols contents and distribution of volatile compounds. Solid-phase micro-extraction (SPME), in combination with gas chromatography/mass spectrometry (GC/MS), were used to study volatile compounds. Thermal stability was evaluated *in situ* by differential scanning calorimetry (DSC). The results obtained show that both refined (R-GPKOG and R-GPKON) and unrefined (GPKOG and GPKON) oils contained high levels of oleic acids (46.36%; 45.89% and 40.18%; 42.46%, respectively), while linoleic and arachidonic acid constituted the major polyunsaturated fatty acids. Among the 11 sterol compounds found in this study, 24-hydroxy-24-methyl cholesterol, clerosterol and sitosterol in GPKON, R-GPKON, GPKOG and R-GPKOG accounted for 68.3, 38.94, 66.33 and 37.71%, respectively. Among the volatile and semi-volatile compounds; Octanal level was the highest in all samples (40-55%) followed by 2, 4-trans, trans-Nonadienal and Hexanal.

INTRODUCTION

Gingerbread plum trees are indigenous, a purely West African species which belongs to Chrysobalanaceae family. These trees produce kernel which is a rich source of both oil and phytosterols and nutritionally important for human health. Non-conventional seeds are being considered due to their constituents that have unique chemical properties and may supply nutritional and functional products (Ramadan and El-Shamy, 2013) when consumed by human beings. There are many worldwide naturally available non-conventional seeds such as gingerbread plum kernels which are found in gingerbread plum trees. Gingerbread plum trees are indigenous, a purely West African species (formerly *Parinari macrophylla* Sabine; now *Neocarya macrophylla* Sabine) Prance belongs to Chrysobalanaceae family (Amza et al., 2011). The kernels were reported to contain 56 and 60% oil in gingerbread plum kernels grown in Niger and Guinea, respectively (Diaby et al., 2016) with high level of oleic acid which makes it different from many other seeds. Several vegetable oils from different sources have been known to contain high percentages of poly- and mono-unsaturated fatty acids but presenting different levels of oleic acid. It was reported that, pumpkin seed oil is composed of an average of 83.74% poly- and mono-unsaturated fatty acids out of which 33.32% linoleic acid, reported by Neđeral et al. (2014). Almont oil contains 88.4 to 92.8% poly- and mono-unsaturated fatty acids with 66.7 to 69.7% as the total percentages of oleic acid (Moayedi et al., 2010). On the other hand, it was reported

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that gingerbread plum kernel oils from Niger and Guinea contained 75.51 and 90.58% poly and monounsaturated fatty acid respectively with 42.46 and 41.43 % oleic acid (Diaby et al., 2016). Foods from plant origin contain several micro-nutrients that contribute health benefits to consumers. Among the plant based nutrients, natural oils contain a number of bioactive lipophilic compounds in which the most interesting ones are the polar lipids, fat soluble vitamins and sterols (Ramadan and El-Shamy, 2013). Sterols can occur in vegetable oils either in free form or esterified with fatty acids and since both fractions have different compositions, the combined determination of these two classes of compounds provides a more informative approach to check vegetable oils authenticity. Cheikh-Rouhou et al. (2008) found that separation of sterol is extremely useful during the refining process whereby the content of free fatty acids is increased while that of that esterified sterol is increased. Volatile compounds play an important role in the organoleptic characteristics of many foods including vegetable oils. Methods applied during the analysis of volatile compounds in vegetable oil involve static headspace, dynamic headspace, and direct chromatography. Such methods have been used for several decades (Wei et al., 2012). However, in recent years' headspace-solid phase micro extraction (HS-SPME) is found to be rapid, solvent-free, and simple method for volatile analysis and has been widely applied in the production of many types of foods including vegetable oils such as corn, soybean, olive, sunflower, and rapeseed oils (Ouni et al., 2011). Volatile compounds can also be used as markers for determining the degree of lipid oxidation, and this can be achieved when oxidized volatile compounds have high correlation with the degree of oxidation and be detected in high amounts (Lee et al., 2007). Despite its relative importance when compared with the conventional oils, gingerbread plum kernel oil has not been fully investigated in relation to its industrial applications.

In this work, gingerbread plum kernel oils have been refined for the first time. The objective was therefore to obtain an informative profile about the chemical nature of fatty acids, phytosterols and flavoring compounds of gingerbread plum kernel oil from Guinea and Niger. Furthermore, the results will be useful for economical and industrial utilization of gingerbread plum kernel.

MATERIALS AND METHODS

Raw materials and oil extraction

Fresh whole gingerbread plum kernels (*N. macrophylla*) were obtained from two different locations (one from Birni N'Gaouré, southern region of Republic of Niger and the other from Gaoul in the Boke region, Republic of Guinea). The kernels were kept dried in desiccators at room temperature. To extract the oils, the kernels were milled using a laboratory scale hammer miller. The resulting paste was dispersed in n-hexane at paste to n-hexane ratio of 1:5 (w/v) and stirred for 15 min at room and let to rest for 2 h. The supernatant which is the mixture of n-hexane and oil was decanted and the cake was re-extracted with n-hexane in the same conditions. This process was repeated until no trace of oil was found in the solvent. Then, the different supernatants were combined and the n-hexane was evaporated using a rotary evaporator at 40°C. The extracted oils were transferred into glass vials, flushed with nitrogen and maintained at -18°C until use.

Chemical analysis

Official methods (Brühl, 1997) were used for the determination of the acid value (method Cd 3d- 63), free fatty acid (method Ca5a-40), peroxide value (method Cd 8-53), iodone value (method Cd 1-25) and saponification value (method Cd 3-25) of the gingerbread plum kernel oils.

Refining process

Although physical refining is more used than chemical refining by now, for the investigations, a chemical refining process was applied in laboratory scale, in order to include a wider range of possible technology steps, starting with the degumming step. At first, hydratable phospholipids were removed by adding 10% w/w deionised water to the oil (85°C, 45 min contact) and, subsequently, the non-hydratable phospholipids were converted in hydratable ones by adding citric acid (3% w/w) to the oil (85°C, 45 min contact) and were also removed. The degummed oil was neutralized by adding 70% excess amount of sodium hydroxide to the oil (90°C, 20 min contact) to form soaps with free fatty acids which were then removed by a four-step washing process each step with 10% (w/w) deionized water (90°C, 20 min contact). In the case of gingerbread plum kernel oil Niger and Guinea two-step neutralization was carried out including the addition of stoichiometric amount of sodium hydroxide at first, followed by adding of a 70% excess amount of this substance. After a drying step at lowered pressure (30–40 mbar) and addition of 60 $mg.kg^{-1}$ citric acid (90°C, 110 min contact), subsequently a bleaching step follow-up was carried out. For this purpose, 0.8% (w/w) (in the case of palm oil: 1.0%) bleaching earth (Tonsil Optimum 214 FF) was added to the oil (90°C, up to 10 mbar) and removed by filtering after 20 min treatment. In the final deodorization step, oil was heated up to 240°C at a pressure of about 1 mbar and small amounts (6 $ml.h^{-1}$) of deionized and degassed water

were added to the oil on the bottom of the deodoriserafte reaching an oil temperature of 160°C. Steam bubbles rise up and different volatile components are stripped by the steam. The temperature is held at 240°C for 20 min and then oil is cooled down. Water addition stops when the oil temperature drops below 160°C by Franke et al. (2009).

Fatty acid analysis of gingerbread plum kernel oil by GC–MS

Fatty acid composition of GPKON and GPKOG was determined according to method prescribed by Amza et al. (2011). The oil was extracted with methyl ether which was prepared directly with the treatment of the oil with sodium methoxide. Gas chromatography/mass spectra (GC/MS) system was used to identify and quantify the fatty acids of the product developed on a FINNIGAN TRACE MS gas chromatograph/mass spectra equipped with a 30 m × 0.25 mm Ov-1701 column. Column flow rate was 0.8 mL /min with helium as the carrier gas, split was 64 mL/min and the source temperature was 270°C. The fatty acid methyl esters were identified by comparison with the retention times of NU CHECK Inc. standards (Elysian, 1L) and quantified by internal normalization.

Sterol composition of gingerbread plum kernel oil

A 150 mg amount of each gingerbread plum kernel refined and unrefined oil was accurately weighed into a clean tube and mixed with 100 µL of dehydrocholesterol solution (2 mg/mL in n-hexane; internal standard) and 400 µL of n-hexane, following the procedure of Fernandes et al. (2015). This mixture was loaded onto a silica SPE column (1 g; Tecnokroma, Spain), conditioned previously with 5 mL of n-hexane (twice). Three 500 µL portion of n-hexane were used to transfer the sample solution to the SPE column. Elution was performed with 5 mL of n-hexane/ethyl acetate (90:10, v/v), followed with more 2.5 mL. Then, 5 mL of ethanol/diethyl ether/n-hexane (50:25:25, v/v/v) were added twice to the SPE column. After solvent evaporation under nitrogen stream of the combined extracts (60°C), 2.5 mL of KOH 1 mol. $L^{¬1}$ in 96% ethanol was added. The solution was heated for 30 min at 70°C. Afterwards, 5 mL of water, 5 mL of diethyl ether (twice), 2 mL of KOH 0.5 mol.$L^{¬1}$ and 4 mL of KCl 0.88% (w/v) were added and centrifuged at 4000 × g for 7 min. The aqueous phase was withdrawn and the organic fraction was dried over anhydrous sodium sulfate. The solution was evaporated under a gentle nitrogen stream at 60°C. Derivatization was performed with 100 µL of N, O-Bis (trimethylsilyl) trifluoroacetamide in 1% trimethylchlorosilane at 70°C for 20 min.

Tocopherols analysis

For the analysis of tocopherols, a solution of 250 mg of oil dissolved in 25 mL of n-hexane was prepared using the method described by Gharby et al. (2016). Then, 20 µL was injected into an HPLC system consisting of a pump (Waters 510; Waters Corp., Milford, MA), an autosampler with a cooling module (Waters 712). Detection was performed using a fluorescence detector (excitation wavelength 290 nm, detection wavelength 330 nm). A normal phase column (hypersil silica, Hewlett Packard) (250 mm × 4.6 mm × 5 µm) was used with hexane/isopropanol 98.5:1.5 (v/v) was used as a mobile phase with the eluent having a flow rate of 2 mL/min.

Differential scanning calorimeter (DSC) of gingerbread plum kernel seed oil

Thermal characteristics of gingerbread plum kernel seed oil were identified using a modulated differential scanning calorimeter (DSC 2920 Modulated DSC-TA Instruments, Newcastle, DE, USA). All the oil samples (2 ± 0.10 mg) were weighed directly into a DSC-pan (SFI-Aluminium, TA Instrument T11024). The seed oil was quickly cooled to -50°C with a speed of 1.5°C/min, maintained for 15 min and heated to 90°C with a heating speed of 15°C/min. The heating operation was repeated twice and the DSC thermographs were recorded during the second melting. The instrument was calibrated for temperature and heat flow using eicosane (Tp = 36.8°C, H = 247.70 J. g¬1) and dodecane (Tp = −9.65°C, H = 216.73 J. g¬1) (Rezig et al., 2012).

Volatile and semi volatile compounds of gingerbread plum kernel seed oils SPME fibers

The SPME fibers were purchased from Supelco (USA). Carboxen/polydimethylsiloxane (thickness 75 µm, CAR/ PDMS) was used in this study. Before being used, new fibers were conditioned in accordance with the recommendations of the producer. Each day, before the samples analyses started, the fibers were thermally cleaned in a GC injector held at 250°C (30 min). Then a blank analysis was performed to verify that no extraneous compounds were desorbed from the fiber.

Optimized SPME procedure

SPME of phthalates was carried out manually, using a PDMS 100 fiber and 20 min extraction from the headspace above 1 g of magnetically stirred (stir position #4, heat position #2, Nuova Stir Plate. Thermolyne, Japan) oil sample modified with 1 ml methanol. The

Table 1. Physicochemical parameter of unrefined and refined gingerbread plum kernel oil.

Oil parameters	GPKON[a]	GPKOG[b]	R-GPKON[c]	R-GPKOG[d]
Free fatty acid (% olei acid)	0.33±0.01[c]	0.34±0.01[c]	0.18±0.01[a]	0.20±0.01[b]
Acid value (mgKOH/g)	0.66±0.02[b]	0.68±0.01[b]	0.35±0.01[a]	0.39±0.01[a]
Peroxide value (meq O2/kg)	41.31±0.03[b]	54.09±0.04[d]	34.65±0.03[a]	48.87±0.03[c]
Iodine value (g I2/100 g)	34.96±0.06[c]	39.13±0.04[d]	31.08±0.07[a]	34.59±0.04[b]
Saponification value (mg KOH/g)	153.35±0.04[a]	162.69±0.07[c]	157.18±0.07[b]	168.48±0.06[d]
Tocopherol (%)				
α Tocopherol	97.88±0.01[d]	62.93±0.03[b]	88.39±0.06[c]	54.29±0.03[a]
β Tocopherol	2.05±0.03[a]	32.37±0.4[b]	3.17±0.03[c]	34.49±0.04[d]
γ Tocopherol	ND	ND	ND	ND
δ Tocopherol	0.08±0.01[a]	4.69±0.4[b]	8.44±0.02[c]	11.22±0.04[d]

Means of three determinations SD; mean values in rows with different letters (a, b, c or d) were significantly different (Turkey's test); significance at ($p < 0.05$) (analysis of variance). [a]GPKON and [c]R-GPKON: gingerbread plum kernel unrefined and refined oil from Niger; [b]GPKOG and [d]R-GPKOG: gingerbread plum kernel unrefined and refined oil from Guinea.

temperature was held at 40°C (measured in headspace) and the sample was incubated for 60 min at 40°C prior to extraction (Holadova et al., 2007).

GC analysis

All desorption's in manual SPME experiments, unless otherwise stated, were performed using a HP 5890 Series II gas chromatograph equipped with a split-splitless injector (liner volume 250 µL), electronic pressure control (EPC) and an electron capture detector (63Ni-ECD) (Hewlett-Packard, USA). A capillary column DB-35 (35% phenyl, 75% polymethylsiloxane) of 30 m length × 0.25 mm i.d. coated with 0.15 µm film (J&W Scientific, USA) was employed for separation of analytes. The data was processed using a GC ChemStation HP 3365 (Hewlett- Packard, USA). The initial oven temperature was set at 45°C for 5 min, increased to 130°C at 20°C.min^{-1}, then increased to 240°C at 3 ^{o}C min−1 and finally increased to 270°C at 20°C.min^{-1} (hold for 2.5 min, total GC run time was 50 min). Nitrogen was used both as carrier and make-up gas at flow rates of 1 and 30 mL.min^{-1}, respectively. The injector and detector temperatures were 250 and 300°C, respectively. In experiments focused on the dynamics and repeatability of PAEs extraction, the manual SPME was combined with gas chromatography coupled with a mass spectrometric detector.

Statistical analyses

All experiments were conducted in triplicate with SPSS Inc. software (version 13.0). One-way analysis of variance (ANOVA) was used to determine significant differences between means, with the significance level taken at a = 0.05. Tukey's honest significant difference (HSD) test was used to perform multiple comparisons between means.

RESULTS AND DISCUSSION

Chemical analysis

Table 1 shows the chemical properties of gingerbread plum unrefined and refined kernel oils. The chemical properties of oils are among the most important characteristics to determine the present condition and quality of oil samples. The amounts of free fatty acids (FFA) in the GPKON and GPKOG samples were 0.33 to 0.34% and acid value (AV) were 0.67 and 0.68 mgKOH/g, respectively. These value lovers than those reported for gingerbread plum kernel oil from Niger by Warra et al. (2013). FFA and AV levels in refined oil samples for R-GPKON and R-GPKOG were the lowest (0.043%, 0.045% and 0.086 mgKOH/g, 0.09 mgKOH/g, respectively). These levels are lower than to those reported for palm unrefined and refined oils (5.97 and 0.21% of palmitic acid respectively) by chemical refining processes (Talukder et al., 2009). Free fatty acid for refined olive oil (0.3% of oleic acid) reported by Santos et al. (2013). The lower FFA contained in the refined vegetable oil, the better the value of the oil. Thus, one of the objectives of refining edible oil is to remove FFA from unrefined oil. In this study, chemical refining was applied and most of the FFA content was eliminated during the neutralization stage. The residual FFA constituted 0.1% of the total fatty acid content. However, some FFA still had to be removed during deodorization to obtain the required final FFA content below 0.043 and 0.045% in R-

GPKON and R-GPKOG respectively. Therefore, optimum conditions should be applied during deodorization in order to keep the FFA content as low as possible. In general, it was observed that FFA content decreased significantly after neutralization step as a result of the influence alkali treatment. However, the levels of FFA did not increase significantly during bleaching by acid activated earth which leads to slight decrease in oil pH. In addition, it was observed that after the deodorization step, the FFA reached the lowest level due to the prolonged heat treatment under vacuum to remove the undesirable compounds including FFA (Warra et al., 2013). Peroxide value measures the concentration of these substances and is often used as an indicator for oil seed quality related to oil oxidation. PV in GPKON and GPKOG ranged from 41.28 to 54.06 mequiv O_2 per kg oil and 34.67 to 48.85 in R-GPKON, R-GPKOG, respectively (Table 1). These values are higher than those in soybean oil (4.57 and 1.10 meq/kg), rapeseed oil (6.03 and 0.85 meq/kg) unrefined and refined respectively by Hua et al. (2016). Oxidation and the formation of peroxides occur during oil extraction and processing and can continue after bottling and during storage. Peroxides are the intermediate oxidation products of oil which lead to the formation of a complex mixture of volatile compounds such as aldehydes, ketones, hydrocarbons, alcohols and esters responsible for the deterioration of organoleptic properties (Gharby et al., 2016). Iodine value is the measure of the degree of unsaturation of oil. The iodine values were 34.9, 39.12, 31.07 and 34.59 I2/100g for GPKON, GPKOG, R-GPKON and R-GPOKOG respectively (Table 1). These values are lower than the value of extra virgin olive oil (87.9 to 86.8 I2/100g) and refined olive oil (88.8 to 73.5 I2/100g) reported by Gharby et al., (2016). Saponification value of gingerbread plum kernel unrefined and refined oils were determined as 153.34, 162.69, 157 and 168 mgKOH/g for GPKON, GPKOG, R-GPKON and R-GPKOG respectively. These values were lower than in virgin olive oil and refined olive oil (188.4 and 205.0 respectively), reported by Gharby et al. (2016).

Fatty acid characterization of gingerbread plum kernel oils

A total of 13 fatty acids were identified in gingerbread plum kernel oil (GKPO) samples. The samples contained 5 kinds of saturated fatty acids (SFA) namely; Palmitic acid (16:0), stearic acid (C18:0), arachidic acid (C20:0), behenic acid (C22:0) and lignoceric acid (C24:0). A total of 8 different kinds of unsaturated fatty acids (USFA) were also identified from the samples out of which 5 were monounsaturated fatty acids (MUFA): palmitoleic acid (C16:1), margaroleic acid (C17:1), oleic acid (C18:1), gadoleic acid (C20:1) and erucic acid (C22:1). The 3 polyunsaturated fatty acids as shown in Table 1 were: (linoleic acid (C18:2), linolenic acid (C18:3) and arachidonic acid (20:4). FA analysis is the most widely practiced analytical technique in lipid science. The different composition of SFA and USFA in GPKO and R-GPKO results into a semi solid state at room temperature. Results revealed that GPKO had high level of palmitic acid: 9.18%, 7.18% (GPKON; GPKOG), 9.46%, 8.1% (R-GPKON; R-GPKOG) and stearic acid 5.21%, 7.81% (GPKON; GPKOG), 4.93%, 5.71% (R-GPKON; R-GPKOG) respectively. The major unsaturated fatty acids as shown in Table 2 are oleic acid: 42.46%, 40.18% (GPKON; GPKOG); 45.89%, 46.36% (R-GPKON; R-GPKOG), linoleic acid: 17.42%, 17.66% (GPKON; GPKOG), 15.30%, 16.88% (R-GPKON; R-GPKOG), arachidonic acid: 17.67%, 21.72% (GPKON; GPKOG); 12.46%, 14.18% (R-GPKON; R-GPKOG) respectively. The percentages for oleic acid, linoleic acid and stearic acid were much higher than those reported for palm oil which accounted for 40, 10 and 5%, respectively (Mba et al., 2015). On the other hand, the percentages for linolenic acid in GPKON and GPKOG were 3.17 and 1.87% respectively, while in R-GPKON and R-GPKOG the percentages were a bit higher (5.28 and 4.23% respectively). Abundant amount of linolenic acid was found in GKPON sample while GKPOG sample was a good source of linoleic, oleic and arachidonic acids (Figure 1). The differences in the distribution of fatty acids is between the two kinds of samples might probably be attributed to differences in geographic and climatic conditions of origin of the two samples. As shown in Table 1, fatty acids such as palmitoleic acid (C16:1), lignoceric acid (18:3), behenic acid (22:0), arachidic acid (20:0) and gadoleic acid (20:1) were present in all samples in a small amounts ranging from 0.04 to 0.97%. The total unsaturated fatty acids content in GPKON, GPKOG, R-GPKON and R-GPKOG (84.08%, 86.27%, 85.43%, 85.23% respectively), was comparable to those reported by Lim et al. (2010) for the value soybean oil (84.6%) and pitaya seed oil (77.22 to 82.01%). Total SFA values in GPKON, GPKOG, R-GPKON and R-GPKOG were 15.92, 16.84, 15.57 and 15.28% respectively. These values are comparable to those values for *Pistacia atlantica* (16.51%) and will almond (10.24%) seed oils, reported by Givianrad et al. (2013). MUFA contents in GPKON, GPKOG, R-GPKON and R-GPKOG (45.82, 41.75, 51.39 and 49.43% respectively), comparable to the value reported for pumpkin seed oil (44.12%) (Rezig et al., 2012). However, the polyunsaturated fatty acids (PUFA) values were 38.26, 41.25, 34.04 and 35.29% for GPKON, GPKOG, R-GPKON and R-GPKOG respectively. The MUFA/PUFA ratio is an important parameter for oil stability in highly unsaturated oils. Thus, regarding fatty acid composition, GPKON and R-GPKON can be considered as more stable oil than other vegetable oils due to their higher MUFA/PUFA ratio.

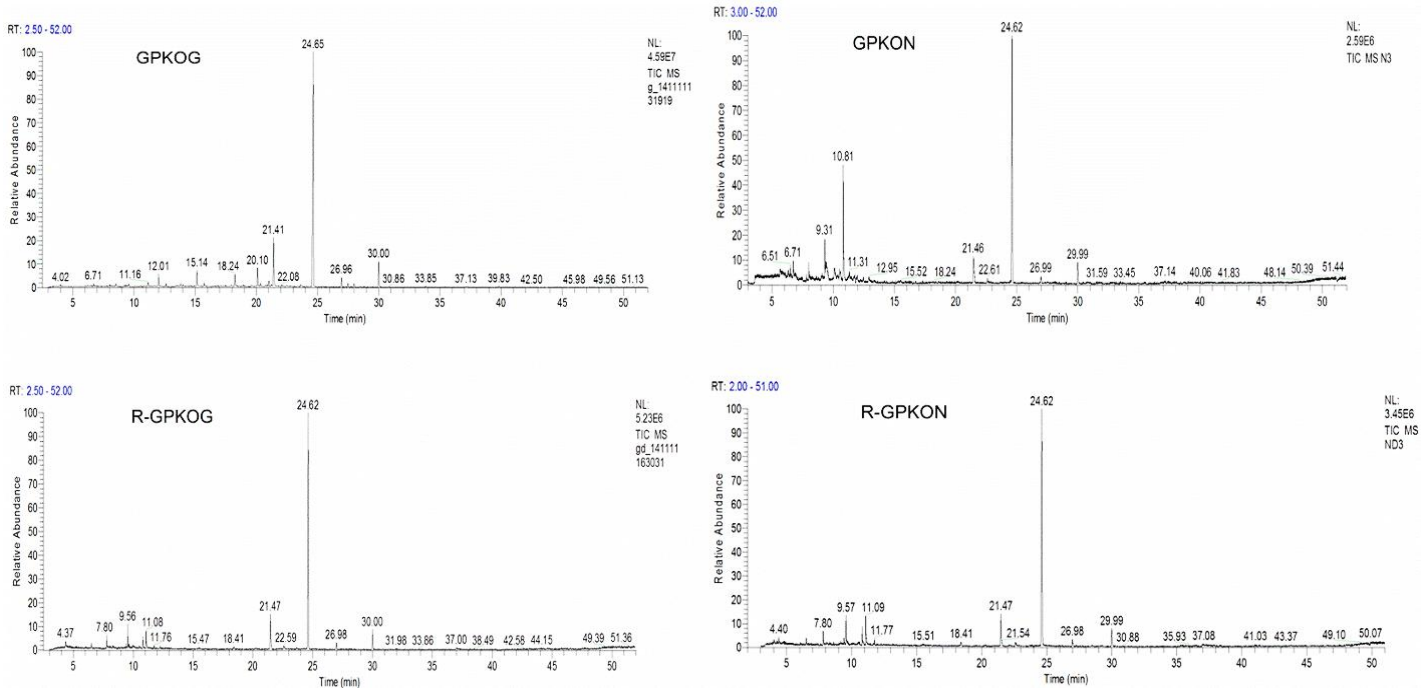

Figure 1. GPKOG, Gingerbread plum kernel unrefined oil from Guinea; **GPKON,** gingerbread plum kernel unrefined oil from Niger; **R-GPKOG,** gingerbread plum kernel refined oil from Guinea; **R-GPKON,** gingerbread plum kernel refined oil from Niger.

Table 2. Fatty acid composition of gingerbread plum kernel oils refined and unrefined (%).

Fatty acid	GPKON[a]	GPKOG[b]	R-GPKON[c]	R-GPKOG[d]
Saturated (SFA)				
Palmitic acid (16:0)	9.19±0.01[c]	7.21±0.04[a]	9.47±0.02[d]	8.11±0.02[b]
Stearic acid (18:0)	5.21±0.03[b]	7.83±0.02[d]	4.92±0.02[a]	5.70±0.01[c]
Arachidic acid (20:0)	0.29±0.03[a]	0.42±0.01[b]	0.33±0.01[a]	0.46±0.02[b]
Behenic acid (22:0)	0.64±0.01[b]	0.83±0.02[c]	0.57±0.01[a]	0.64±0.2[b]
Lignoceric acid (24:0)	0.59±0.02[c]	0.63±0.02[c]	0.28±0.02[a]	0.38±0.02[b]
Monounsaturated (MUFA)				
Palmitoleic acid (16:1)	0.55±0.02[c]	0.47±0.02[b]	0.34±0.02[a]	0.30±0.02[a]
Margaroleic acid (17:1)	0.07±0.01[a]	0.05±0.01[a]	0.31±0.02[b]	0.96±0.01[c]
Oleic acid (18:1)	42.45±0.02[c]	40.19±0.02[a]	45.89±0.02[c]	46.36±0.02[d]
Gadoleic acid (20:1)	0.50±0.02[b]	0.54±0.01[b]	0.44±0.02[a]	0.50±0.03[b]
Erucic acid (22:1)	2.25±0.02[c]	0.52±0.01[a]	4.43±0.02[d]	1.35±0.02[b]
Poly unsaturated (PUFA)				
Linoleic acid (18:2)	17.44±0.03[c]	17.65±0.02[d]	15.31±0.05[a]	16.87±0.02[b]
Linolenic acid (18:3)	3.19±0.02[b]	1.87±0.05[a]	5.29±0.03[d]	4.22±0.04[c]
Arachidonic acid (20:4)	17.66±0.01[c]	21.74±0.03[d]	12.44±0.04[a]	14.20±0.04[b]
SFA	15.92±0.1[a]	16.92±0.11[c]	15.57±0.08[c]	15.29±0.09[b]
MUFA	45.82±0.09[a]	41.77±0.07[d]	51.41±0.1[b]	49.47±0.1[c]
PUFA	38.29±0.06[c]	41.26±0.1[d]	33.06±0.12[b]	35.29±0.08[a]
TUFA	84.11±0.15[c]	83.03±0.08[b]	84.47±0.13[d]	84.76±0.18[b]
MUFA/PUFA	1.19±0.3	1.01±0.28	1.6±0.62	1.4±0.89

Means of three determinations SD; mean values in rows with different letters (a, b, c or d) were significantly different (Turkey's test); significance at ($p < 0.05$) (analysis of variance). [a]GPKON and [c]R-GPKON: gingerbread plum kernel unrefined and refined oil from Niger; [b]GPKOG and [d]R-GPKOG: gingerbread plum kernel unrefined and refined oil from Guinea.

Table 3. Sterol composition of gingerbread plum kernel oil refined and unrefined (%).

Compound	GPKON[a]	GPKOG[b]	R-GPKON[c]	R-GPKOG[d]
24-Hydroxy-24-methyl cholesterol	39.35±0.02[c]	41.46±0.04[d]	22..45±0.02[b]	19.46±0.04[a]
24-Dihydrolanosterol	4.83±0.03[d]	3.53±0.02[c]	2.22±0.03[a]	3.33±0.02[b]
Sitostanol	3.68±0.02[d]	2.85±0.02[c]	2.48±0.02[b]	1.13±0.02[a]
β-Amyrin	1.30±0.02[c]	1.72±0.03[d]	0.73±0.02[a]	0.87±0.03[b]
Δ5-avenasterol	4.46±0.03[d]	6.73±0.02[c]	4.07±0.03[b]	2.28±0.03[a]
24-Hydroxycholesterol	6.75±0.02[d]	4.51±0.02[c]	2.41±0.02[a]	3.45±0.02[a]
Campesterol	4.84±0.01[d]	2.40±0.02[b]	3.34±0.03[c]	1.37±0.02[a]
Clerosterol	12.78±0.02[d]	11.62±0.02[c]	7.33±0.02[a]	8.66±0.01[b]
Sitosterol	16.19±0.02[d]	13.17±0.03[c]	9.15±0.01[a]	9.64±0.02[b]
Phytol	2.31±0.02[b]	4.52±0.02[d]	1.79±0.03[a]	2.38±0.03[c]

Means of three determinations SD; mean values in rows with different letters (a, b, c or d) were significantly different (Turkey's test); significance at ($p < 0.05$) (analysis of variance). [a]GPKON and [c]R-GPKON: gingerbread plum kernel unrefined and refined oil from Niger; [b]GPKOG and [d]R-GPKOG: gingerbread plum kernel unrefined and refined oil from Guinea.

However, the MUFA/PUFA values in GPKON, GPKOG, R-GPKON, R-GPKOG (1.19%, 1.01%, 1.5%, 1.4% respectively) based on this study are much lower than those for almond species oils, AZ (3.86), AH (3.24) and AJ (3.34) (Moayedi et al., 2010).

Sterol composition of gingerbread plum kernel oil

Sterols are important constituents of oils due to their antioxidant activity and impact on human health. As presented in Table 3, eleven sterol related compounds were detected in the samples. Among the sterols, 24-Hydroxy-24-methyl cholesterol was identified as a sterol marker since its observed level was highest (41.55, 39.34, 22.47 and 19.43% in GPKOG, GPKON, R-GPKON and R-GPKOG, respectively). The next major sterol compounds were sitosterol and clerosterol. Plus, the sterol marker, these three major compounds accounted for more than 68.3 %, 66.33 %, 38.94% and 37.71% in (GPKON, GPKOG, R-GPKON and R-GPOKG), respectively of total sterol content other sterol components detected were cholesterol, 24-dihydrolanosterol, campesterol, clerosterol, sitosterol 24-hydroxycholesterol, β-amyrin and phytol and their levels were less than 8 and 10% in refined and unrefined oil respectively. The observed values of sitosterol in both the refined and unrefined oils were almost equal since their total content was approximately 9.63 and 9.16% in R-GPKOG and R-GPKON, respectively. Among the different phytosterols, sitosterol has been most intensively investigated with respect to its beneficial and physiological effects on human health (Cheikh-Rouhou et al., 2008). The levels of sitosterol in GPKON, GPKOG, R-GPKON and R-GPKOG were 16.18, 13.17, 9, 16 and 9.63%, respectively. These levels are lower than those reported for wild pistachio and wild almond seed oil 87.73 and 91.32% respectively, by Givianrad et al. (2013). The values for Δ^5–avenasterol in GPKON, GHPKOG R-GPKON and R-GPKOG were 4.47, 6.73, 4.07 and 2.29% respectively, while those for Campesterol in the same samples 4.82, 2.4, 3.33 and 1.36%, respectively. Campesterol and sitosterol are generally found in high levels in plant oils and are structurally very similar to cholesterol except the side chain on C17 whereas Δ^5–avenasterol is a derivative of sitosterol. The Percentage difference between the two samples in our results might be attributed to essentially geographical conditions, climate and seed maturation. However, as sterols are affected by processing; about 40% of these components can be removed from the oil during deodorization.

Tocopherol composition of gingerbread plum kernel oil

The tocopherol values of gingerbread plum kernel oils from Niger and Guinea oils were determined by the high performance liquid chromatography (HPLC), and given in Table 1. Tocopherols are important components of the unsaponifiable fraction in vegetable oilseed. In addition to fatty acids, kernel oils are excellent sources of vitamin E. Tocopherols are biologically highly active natural antioxidants. Three tocopherols were found, and α–tocopherol was the major component, constituting 97.89, 62.96, 88.39 and 54.26% in GPKON, GPKOG, R-GPKON and R-GPKOG respectively, followed by β–tocopherol and δ–tocopherols in both samples (Table 1). γ–Tocopherol was not detected in the present study. α–tocopherol is the major tocopherol present in the virgin olive oil and refined olive oil 91.3 and 82.3% respectively

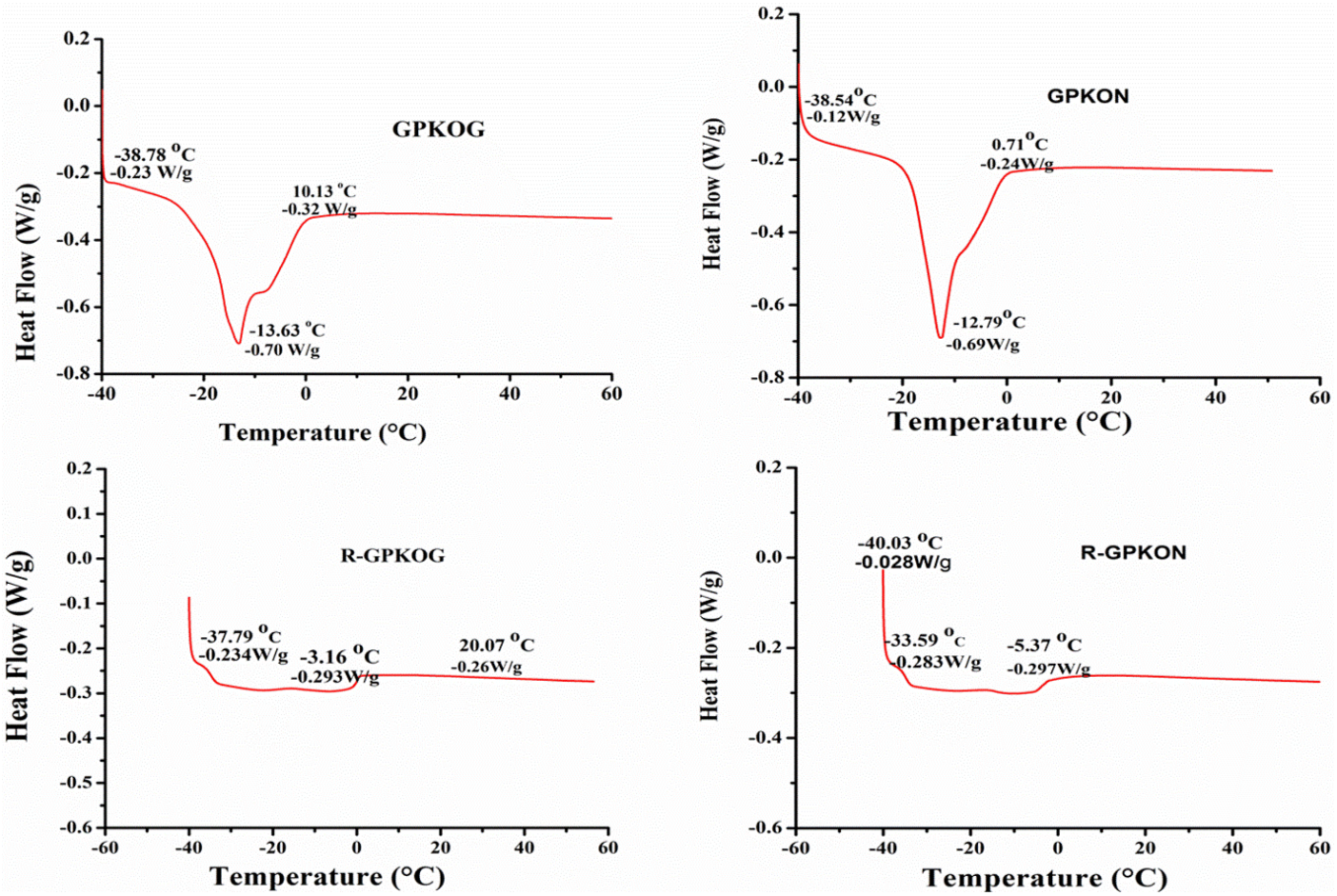

Figure 2. GPKOG, Gingerbread plum kernel unrefined oil from Guinea; **GPKON,** gingerbread plum kernel unrefined oil from Niger; **R-GPKOG,** gingerbread plum kernel refined oil from Guinea; **R-GPKON,** gingerbread plum kernel refined oil from Niger.

by Gharby et al. (2016). α–Tocopherol affects human nutrition and health aspects.

Differential scanning calorimetry (DSC) of gingerbread plum kernel oil

DSC determination was performed to unrefined and refined gingerbread plum kernel oils (Figure 2). The samples R-GPKOG and R-GPKOG exhibited four transitions when heated from -60 to 0°C and -60 to 60°C respectively. The first and the last transitions for R-GPKOG and R-GPKOG occurring at -37.79°C; 0°C and -33.59°C; 0°C, respectively. The samples GPKOG and GPKON exhibited four transitions when heated from -40 to 0°C and -40 to 60°C respectively. The first and the last transitions in GPKOG and GPKON occurred at approximately same temperatures -38.78°C; 0°C and -38.54°C; 0°C, respectively. The complex endothermic events occurring at higher temperatures were attributed to the melting of crystallized lipids and were characterized by multiple overlapping (Rezig et al., 2012) contributions as previously observed in vegetable and olive oils. No endothermic phenomenon was observed beyond 0°C. This property a might confirm the liquid state of seed oil at room temperature (25°C). The DSC heating curve as shown in Figure 2 indicates the occurrence of endothermic peaks at -12.79, -13.63, -33.59 and -37.79°C, corresponding to the melting points of GPKON, GPKOG, R-GPKON and R-GPKOG respectively. The melting point of R-GPKON (-33.59°C), R-GPKOG (37.79°C) as shown in Figure 2 was lower than that of soybean oil (-16°C). A lower melting point is desirable in terms of nutrition (Nehdi et al., 2014). Actually, the thermogram seemed to correspond to a number of components higher than the visible ones, which suggested the presence of triglyceride fractions with melting points too near to be differentiated under the conditions applied.

Table 4. Volatile and semi-volatile compounds of gingerbread plum kernel oils from Guinea and Niger.

S/N	Compounds	GPKON[a]	GPKOG[b]	R-GPKON[c]	R-GPKOG[d]
	Aldehydes (10)				
1	2,4-Deadienal (E,E)-	0.99	0.74	0.61	0.45
2	Pentanal	2.42	2.05	1.87	1.17
3	Acetaldehyde	1.73	0.19	0.47	0.32
4	Cis-hept-2-enal	0.49	0.29	0.89	0.47
5	2,4-Nonadienal	3.87	1.22	3.09	2.45
6	2,4-trans-trans-Nonadienal	17.92	15.35	2.64	1.41
7	2-Undecenal	7.08	4.47	1.72	1.34
8	Octanal	40.34	54.68	46.15	53.49
9	Hexanal	10.68	13.17	14.49	15.68
10	Nonanal	0.59	0.47	1.67	1.28
	Alcohols (4)				
11	n-Heptanol	2.08	1.5	1.63	0.4
12	2-Butanol	1.08	2.56	1.37	3.52
13	3-Pentanol	0.62	0.57	0.78	0.45
14	1-(Methylthio)-2-propanol	0.86	1.03	0.16	0.52
15	**Ketones (2)**				
16	Acetophenone	0.75	0.67	0.63	0.46
17	2,4-Diethylpentan-3-one	2.15	1.94	2.31	0.66
	Terpenes (2)				
18	Limonene	0.56	0.23	0.76	0.28
19	Cis-Ocimne	0.45	0.2	0.37	0.26
	Aromatics (6)				
20	1-Methyl naphtalene	3.97	0.66	4.45	0.39
21	1,2-Diphenylethylene	0.98	0.91	1.28	0.36
22	2-Methyl naphtalene	3.22	0.26	5.31	0.14
23	2-Methylpyrazine	6.47	7.55	7.81	8.08
24	2-Aacetylpyrrole	0.75	0.47	0.87	0.59
25	Styrene	1.28	1.03	0.85	0.4
	Acids(2)				
26	Hexanoic acid	1.5	0.54	2.17	1.25
27	Acetic acid	2.64	4.57	1.37	3.52
	Alkanes (2)				
28	Hexane	2.3	2.05	1.63	0.4
29	Octane	0.67	0.2	1.89	0.47
	Ether (1)				
30	2-Methyl furan	0.52	0.26	1.15	0.48
	Phenolic (1)				
31	Phenol,2,4-di-ter-butyl	0.54	0.2	2.07	1.34

[a]GPKON and [c]R-GPKON: gingerbread plum kernel unrefined and refined oil from Niger; [b]GPKOG and [d]R-GPKOG: gingerbread plum kernel unrefined and refined oil from Guinea.

Volatiles and semi volatiles compounds content in gingerbread plum oil

Volatile compounds such as poly-dimethylsiloxane (PDMS), poly-acryrate, PDMS/divinyl-benzene (DVB), carbowax/DVB and DVB/carboxen/PDMS are incorporated into materials for coating fibers. In this study, we chose PDMS since it is recommended source of volatiles and a relatively non-polar compounds (Chen et al., 2007). Using SPME-GC/MS, a total of thirty volatile compounds were identified in both unrefined and refined gingerbread kernel oil. These volatile compounds as presented in Table 4 belonged to different chemical groups such as: Alkanes (2), aldehydes (10), ketones (2),

terpenes (2), aromatic (6), phenolic (1), alcohols (4), ether (1), acids (2) and furan (1). We further found that the volatile compounds of all samples were identical and included acetic acid hexanoic acid. The amounts of acetic acid that were in GPKON, R-GPKON, GPKOG, R-GPKOG were 2.64%, 4.57%, 1.37%, 3.52%, respectively, while those for hexanoic acid were 1.5%, 0.54%, 2.17%, 1.25%. Previous reports indicate that these acidic volatile compounds are well known for giving off a sour, pungent fatty and sweaty aroma (Bail et al., 2008). It was also noted that every sample was degraded due to oxidation, preservation and storage conditions resulting into generation of flavour active carbonyls such as, hexanal, 2-heptenal and 2,4,6-trimethoxyacetophenone. Of these components, the content of 2-heptenal was high in the refined samples (54.68%, 53.49% in R-GPKON and R-GPKOG respectively). Two major oxidized volatiles from FFA were 2-heptenal and hexanal. Hexanal is a typical volatile compound from linoleic acid at 13-hydroperoxide and this compound is an indicator for measuring lipid oxidation in foods (Lee et al., 2007). In comparison with other vegetable oils, GPKO contained higher hexanal levels 10.69%, 13.17%, 14.49%, 15.68% in GPKON, R-GPKON, GPKOG, R-GPKOG respectively, than other vegetables oils such as grape seed oil whose reported content occurs in the range of 1.4 to 7.5% (Bail et al., 2008). A total of 10 aldehydes consisting of saturated and unsaturated compounds were identified in GPKO. The aldehydes particular 2-alkenals and 2,4-alkadienals arose from lipid oxidation of the mono and polyunsaturated fatty acids while nonanal, octanal, 2-alkenals arose from oleic acid oxidation. On the other hand, acetaldehyde, pentanal, hexanal, 2-heptenal, 2,4-nonadienal, 2,4-decadienal, (E, E)-,2,4-trans, trans-nonadienal arising from linoleic acid oxidation. Most of the ketones except 2,4-diethylpentan-3-one and acetophenone were present in all samples. A total of four alcoholic compounds were identified in all samples and these are; n-heptanol, 2-butanol, 3-pentanol and 1-(Methylthio)-2-propanol. Heptanol has odor detection thresholds and can therefore exert an influence on the aroma profile of kernel oils. The levels of 1-(Methylthio)-2-propanol identified in GPKON, R-GPKON, GPKOG, R-GPKOG were 0.86%, 1.03%, 0.16%, 0.52% respectively. Since1-(Methylthio)-2-propanol is a sulfur-containing compound, its formation may be associated with levels of free cysteine and methionine. Reports from previous studies indicate that this compound occurs in the range of 0.151 and 0.189 g perevery 100 g of almonds (Xiao et al., 2014). We also identified 2 terpenes compounds in all samples, these are; Cis-Ocimene and limonene. These terpenes are known to be naturally occurring smell intense compounds in fatty products and may be used to differentiate between types of oils. Six aromatic compounds namely 1-methylpyrazine, 2-methylpyrazine, 1,2-diphenylethylene, 2-acetylpyrrole and styrene plus an ether compound identified as 2-methyl furan were also identified in all samples. The distribution of oleic acid in GPKON, R-GPKON, GPKOG, R-GPKOG was 42.26%, 41.42%, 44.89%, 45.36% respectively; while that of linoleic acid was 17.43%, 18.67%, 16.3%, 19.4% respectively. Therefore, almonds contain more oleic acid (63–78%) than GPKON and GPKOG. Moreover, linoleic acid is a precursor for many aldehydes and alcohols including (E)-2-heptenal and nonanal and sensitive to lipid oxidation. Phenolic compound in particular; phenol 2,4-di-tert-butyl was identified in the samples and this compound is responsible for a smoky, woody and phenolic note. Hydrocarbons such as hexane and octane were identified in the samples. However, these hydrocarbons do not have a significant impact on the aroma and flavor profile of hydrocarbons (alkanes-50 and alkenes-18) due to their small molecular size.

Conclusion

The findings in this study have revealed that gingerbread plum kernel oils are a rich source of many nutrients that appear to have a very positive effect on human health. The oil is characteristically distinguished from other kernel oils due to its high oleic acid. However, higher hexanal and linoleic acid content in gingerbread plum kernel oil renders it unstable and therefore, further processing step is required for improving desirable characteristics of the oil including its shelf life. Furthermore, results have revealed the occurrence of high levels of hydroxy-24-methyl cholesterol, sitosterol and clerosterol. The presence of phytosterols may bring nutritional and functional benefits to food systems. The finding of this study reflects the indication of the potentially economical utility of these seeds as a new source of edible oils. This study consolidates the possibility of incorporating gingerbread plum kernel oils into food, cosmetics and pharmaceutical products.

ACKNOWLEDGEMENTS

This project was funded by Jiangsu Provincial Natural Science Foundation (BK20140149) and the Fundamental Research Funds for the Central Universities (JUSRP11439).

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