GRASAS Y ACEITES 67 (1)
January–March 2016, e117
ISSN-L: 0017-3495
doi: http://dx.doi.org/10.3989/gya.0362151

Physicochemical and antioxidant characteristics of gingerbread plum (*Neocarya macrophylla*) kernel oils

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Submitted: 10 March 2015; Accepted: 21 September 2015

SUMMARY: This study examined the physicochemical characteristics and antioxidant activities of oils extracted from gingerbread plum kernels grown in two different areas (Niger and Guinea). The oil contents were found to be significantly different (P≤0.05); 56% and 60% for gingerbread plum kernels originated from Niger (GPKN) and guinea (GPKG), respectively. GPKG showed the highest levels for physical parameters such as iodine, saponification, free fatty acid, acid and peroxide values when compared with GPKN. The major monounsaturated fatty acid in both GPKN and GPKG was oleic acid (42.46 and 41.43%, respectively) while the polyunsaturated fatty acids consisted of linoleic and arachidonic acid. Arachidonic acid was at higher levels (17.67 and 21.72% in GPKN and GPKG, respectively) than those in common vegetable oils. Results from antioxidant activity essays showed that GPKG is more active than GPKN in DPPH radical scavenging, β-carotene and phenolic contents while GPKN showed the highest values for reducing power and flavonoid. Of the 11 sterol compounds found in this study, 24-hydroxy-24-methyl cholesterol, clerosterol and sitosterol accounted for 68.5% and 66.33% in GPKN and GPKG, respectively. Finally, all tocopherol vitamers (except γ-tocopherol) were present in GPKN and GPKG with α-tocopherol being the main element in both samples.

KEYWORDS: *Antioxidant activity; Fatty acid profile; Gingerbread plum kernel; Oil indices; Sterols; Tocopherols*

RESUMEN: ***Características físico-químicas y antioxidantes del aceite de semillas de* Neocarya macrophylla**. En este estudio se examinaron las características físico-químicas y actividad antioxidante de los aceites de semilla de *Neocarya macrophylla* procedentes de dos regiones distintas, Níger y Guinea. Los contenidos grasos de las dos semillas resultaron ser significativamente (P≤0.05) diferentes: 56% para las semillas procedentes de Níger (GPKN) y 60% para las de Guinea (GPKG). El aceite de semillas de Guinea mostraba valores de parámetros químicos tales como índices de iodo, saponificación, peróxidos, y acidez, más elevados que el aceite de semillas de Níger. El ácido oleico resultó ser el ácido graso mono-insaturado más abundante en ambos casos (42.26% para el aceite de Níger y 41.42% para el de Guinea), mientras que los ácidos grasos poli-insaturados predominantes resultaron ser los ácidos linoleico y araquidódico. Los niveles de ácido araquidónico (17.68% para GPKN y 21.72% para GPKG) resultaron más elevados que los encontrados en otros aceites vegetales comunes. El aceite de GPKG demostró mayor capacidad de eliminación de radicales (ensayo de la difenil picrilhidrazina), así como mayores contenidos de β-caroteno y fenoles, mientras que el de GPKN mostró mayor poder reductor y contenido de flavonoides. De los 11 esteroles detectados, 24-hidroxi-24-metil colesterol, colesterol y sitosterol representaban el 68.5% y el 66.33% para los aceites de GPKN y GPKG respectivamente. Por último, todos los tocoferoles (excepto γ-tocoferol) estaban presentes en los dos aceites, siendo α-tocoferol el más abundante.

PALABRAS CLAVE: *Actividad antioxidante; Esteroles; Índices de aceites;* Neocarya macrophylla*; Perfil de ácidos grasos; Tocoferoles*

Citation/Cómo citar este artículo: Diaby M, Amza T, Onivogui G, Zou X-Q, Jin Q-Z. 2016. Physicochemical and antioxidant characteristics of gingerbread plum (*Neocarya macrophylla*) kernel oils. *Grasas Aceites* **67** (1): e117. doi: http://dx.doi.org/10.3989/gya.0362151.

1. INTRODUCTION

Currently, the world's vegetable oil demand (estimated as high as 145 MT) is rapidly growing due to human population growth and the expanding oleochemical industry, thus prompting the need to search for newer and under-utilized non-conventional vegetable oil sources (Anwar *et al*., 2014a; Anwar *et al*., 2014b). Oil World publications (Mielke, 2001) recognize 17 commodity oils, of which four are of animal origin and the remainder is from vegetable sources. Within the sources of vegetable oils, it is useful to distinguish three different types: by-products such as cotton and corn, which are grown primarily for fiber and cereal, respectively, while the oil is a by-product; tree crops, which include palm, palm kernel, coconut and olive oils; and annual crops, such as rape, sunflower and linseed oils. Gingerbread plum kernel oils can be classified within the tree crop source and are native to Western Africa and Central America including Ghana, Guinea, Guinea Bissau, Ivory Cost, Liberia, Mali, Niger, Nigeria, Senegal, Sierra Leone, Gambia and Panama.

The gingerbread plum is a tree of up to 10 m high, although often less, and belongs to the Chrysobalanaceae family. It is also known by two other names, *Neocarya* macrophylla (Sabine) Prance and *Parinari macrophylla Sabine* (Frederick, 1961). The tree produces fruits in the form of an ellipsoid drupe, glabrous, yellowish-brown with grey warts on the surface, 4–5 cm long and 2.3–3.5 cm across, with an endocarp embedded in a thick pulp (Fig. 1a). The flesh is soft and yellowish when fresh, with a peculiar flavor sometimes likened to avocado. The endocarp contains one or two kernels (Arbonnier, 2004). The ratio of the endocarp to the kernel is about 85: 15%.

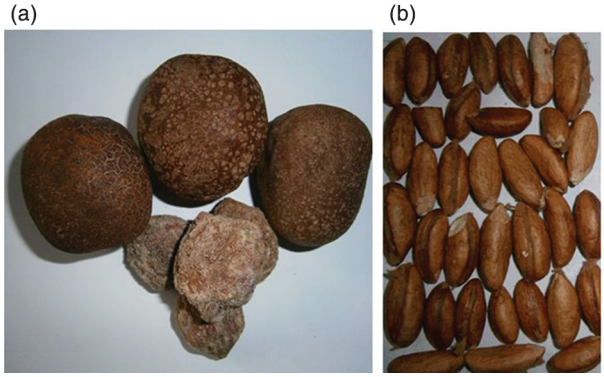

FIGURE 1. Gingerbread plum (*Neocarya macrophylla*) (a): Fruits and endocarps (b): Kernels.

Gingerbread plum kernel (Fig. 1b) is edible and has been recorded as containing 62% oil, while 9% has been found in the endocarp (Kerharo and Adam, 1974). The kernels are usually roasted and enjoyed like cashews or almonds. Some are consumed as snacks, others mixed into cooked dishes, and a few are pressed to yield cooking oil (Amza *et al*. 2010). Therefore, identifying new opportunities for the use of gingerbread plum kernels is more than necessary.

An overview of selected works carried out on gingerbread plum (Frederick, 1961; Amza *et al*. 2010; Audu *et al*., 2005; Mann *et al*., 2009) revealed that there is not a complete study on the physicochemical characteristics of gingerbread plum kernel oils in the literature, suggesting the potential for development of value-added products from these underutilized oilseeds. Therefore, a thorough investigation on the chemical composition and antioxidant activities of gingerbread plum kernel oils will contribute significantly to the current limited data available on these lesser-known oilseeds as potential raw materials for their application in food products.

In the present study, the physicochemical characteristics and some antioxidant activities of gingerbread plum kernel oils from two different geographical locations (Niger and Guinea) were investigated. Physicochemical parameters include pH, color, oil indices (iodine value, saponification value, free fatty acid, acid value, and peroxide value); fatty acid, sterol and tocopherol compositions. In addition, DPPH radical scavenging activity, reducing power, phenolic content, flavonoid and β-carotene were also evaluated.

2. MATERIALS AND METHODS

2.1. Raw materials and oil extraction

Fresh whole gingerbread plum kernels (*Neocarya 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 a paste to n-hexane ratio of 1:5 (w/v) and stirred for 15 min at room temperature and let stand 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.

2.2. Physical parameters

IV, SV, FFA, AV, PV, color and pH were the indices measured for the extracted oils. FFA, IV and SV were determined according to the methods Ca5a-40, Cd 125 and Cd 3-25, respectively, from the AOCS Official Methods of Analysis (AOCS, 1993a; AOCS, 1993b; AOCS, 1993c). The PV was determined by method Cd 8-53 of the AOCS Official Methods of Analysis (AOCS, 1998) and AV was determined based on the AOAC Official Methods of analysis (AOAC, 1998). To determine the color parameters, a Hunter Lab digital colorimeter was used to measure gingerbread plum kernel oil's color and color scales L*a*b* values were recorded.

2.3. Fatty acid analysis by GC–MS

The fatty acid composition of gingerbread plum kernel oil from Niger (GPKON) and gingerbread plum kernel oil from Guinea (GPKOG) was determined as reported by Amza *et al.* (2010). Fat was extracted with methyl ether which was prepared directly with the treatment of the fat with sodium methoxide. A 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. The column flow rate w as 0.8 $mL \cdot min^{-1}$ with helium as the carrier gas, the split was 64 $mL \cdot min^{-1}$ 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.

2.4. Antioxidant activity

2.4.1. DPPH radical-scavenging activity

The scavenging activity of GPKON and GPKOG on DPPH was determined using the method described by Mensor (2001) with slight modification. This method depends on the reduction of purple DPPH to a pale yellow colored diphenyl picrylhydrazine. The determination of the disappearance of free radicals was made using a spectrophotometer. The remaining DPPH, which showed maximum absorption at 517 nm, was measured. The samples were prepared at different concentrations (0.2, 0.4, 0.6, 0.8, and 1 $mg \cdot mL^{-1}$) using ethanol (95%). 0.5 mL of DPPH (0.3 mM) were added to 2.5 mL of the different concentrations. These are test solutions (A_1). 1 mL of ethanol (95%) was added to 2.5 mL of the various concentrations. These are blank solutions (A_0). 1 mL of 0.3 mM DPPH and 2.5 mL ethanol were used as a negative control (A_2). As DPPH is sensitive to light, it was exposed to the minimum possible light. These solutions were allowed to react at room temperature for 30 min. The absorbance values were measured at 517 nm and results were determined using the following equation:

$$DPPH\ scavenging\ activity\ (\%) = 1\left(\frac{A_1 - A_0}{A_2}\right) \times 100$$

2.4.2. Reducing power

The reducing power was determined according to Oyaizu (1986). 2.5 mL of sample (0.2, 0.4, 0.6, 0.8, and 1 $mg \cdot mL^{-1}$) were mixed with a phosphate buffer (2.5 mL, 0.2 M, pH 6.6) and potassium ferricyanide [$K_3Fe(CN)_6$] (2.5 mL, 1%). The mixture was incubated at 50 °C for 20 min. A portion (2.5 mL) of trichloroacetic acid (10%) was added to the mixture to stop the reaction, which was then centrifuged at 2000 rpm for 10 min. The upper layer of solution (2.5 mL) was mixed with distilled water (2.5 mL) and $FeCl_3$ (500 µL, 0.1%) and the absorbance was measured at 700 nm. An increased absorbance of the reaction mixture indicated increased reducing power.

2.5. Determination of total phenolic content (TPC)

A standard solution was prepared using a gallic acid solution at a concentration of 0.1–1.0 $mg \cdot mL^{-1}$. The reaction mixture is composed of 50 µL of standard or sample solution, 200 µL of freshly prepared Folin–Ciocalteau reagent, and 3 mL of distilled water. The mixture is left at room temperature for 10 min and 500 µL of 20% sodium carbonate was added. The solution was mixed and incubated in water bath at 40 °C for 20 min and the reaction was stopped in an ice bath. The absorbance was measured at 765 nm and distilled water was used as a blank (Zhou and Yu, 2004). TPC contents were quantified and expressed as Gallic Acid Equivalent (GAE) from a calibration curve; $y=0.0095x\pm0.046$ ($R^2=0.9883$).

2.6. Estimation of total flavonoid content

Rutin is used as standard for the estimation of total flavonoids. 10 mg of rutin were dissolved in 10 mL of methanol and a calibration curve was made from

aliquots of 0.1, 0.2, 0.4, 0.6, 0.8 and 1.0 mL from the above stock solution. To each concentration, 1.5 mL of methanol, 0.1 mL of 10% aluminum chloride, 0.1 mL of 1M potassium acetate and 2.8 mL of distilled water were added. The reaction mixture was kept at room temperature for 30 min and the volume was made up with water. The absorbance of the resulting solutions was measured at 415 nm against a blank. The calibration curve was prepared by plotting absorbance Vs concentration and it was found to be linear over this concentration range of 10–100 $\mu g \cdot mL^{-1}$. The samples (1 $m \cdot mL^{-1}$) were prepared in the same conditions as described above. The concentration of total flavonoids in the test samples was determined by extrapolation from the calibration curve. The total flavonoid content was expressed as $\mu g \cdot mL^{-1}$.

2.7. Determination of β–carotene

β-carotene was isolated and quantified as described by Gimeno *et al*. (2000). 400 mg of sample were inserted into tubes with a screw-on top and 0.2 g of ascorbic acid, 15 mL of absolute ethanol and 4 mL of potassium hydroxide (76% m/V) were added for the saponification process, which was performed under nitrogen atmosphere. The tubes were continuously shaken for 30 min at 70 °C and the samples were then cooled in an ice bath and 5 mL of NaCl (2.5% m/V) were added to provoke phase separation. A mixture of n-hexane/ethyl acetate (85:15 v/v) was also added in order to extract the non-saponifiable fraction. The organic phases obtained were evaporated to dryness at 40 °C using a rotor vapor. The residue was then re-dissolved in methanol. In this process, the chromatographic injection must be performed as early as possible to avoid the oxidation and decomposition of the β-carotene. Alternatively, the sample can be stored at −20 °C for a maximum of one week.

2.8. Sterol analysis

For the preparation of unsaponifiable matter, mainly sterols; each oil sample (400 mg) was mixed with KOH (10%) in methanol (50 mL) using sonication and horizontally shaken at 30 °C for 24 h. After incubation, distilled water (10 mL), ethanol (1 mL) and hexane (20 mL) were added. The samples were vigorously shaken in separatory funnels and after partition, the hexane layer was collected. The remaining aqueous/alcohol phase was re-extracted twice with hexane (20 mL). The hexane extracts were pooled and dried over anhydrous $MgSO_4$ and the solvent removed in vacuo. The dry extracts were suspended in dichloromethane (10 mL). Prior to GC-MS, 50 mL (dried under nitrogen) of these suspensions were derivatized with bis (trimethylsilyl) trifluroacetamide (BSTFA) (100 µL) at 37 °C for 15 min.

2.9. Analytical HPLC determination of tocopherols

Normal phase HPLC with fluorescence detection (excitation 292 nm, emission 325 nm) was used to analyze tocopherols (Kamal-Eldin *et al*., 2000). The HPLC system consisted of a pump (Waters 510; Waters Corp., Milford, MA), an auto-sampler with a cooling module (Waters 712), a scanning fluorescence detector (Waters 474), and an Inertsil silica column (5 mm, 250 mm* 4.6 mm; Varian Chromapack, Middelburg, Netherlands) with a silica guard column (Guard-Pak Silica, Waters). The temperature of the column oven was 30 °C. Separation of the vitamers was based on isocratic elution. The mobile phase contained 3% 1, 4-dioxane and 97% n-hexane. The flow rate of the mobile phase was 2 $mL \cdot min^{-1}$. Tocopherols were quantified with an external standard method in which quantification was based on peak areas. The method was validated by determining the following parameters: detection and determination limits, range of linearity and repeatability. Detection limits were defined as a signal three times the height of the noise. Determination limits were defined as three times the detection limit.

2.10. Statistical Analysis

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 among means, with the significance level taken at a=0.05. Tukey's HSD test was used to perform multiple comparisons among means.

3. RESULTS AND DISCUSSION

3.1. Oil Content and Physical Characteristics

3.1.1. Oil Contents

The results for the oil contents of gingerbread plum kernel oil from Niger (GPKON) and gingerbread plum kernel oil from Guinea (GPKOG) are presented in Table 1. The oil level in GPKON was lower than that in GPKOG, 56.15% and 60.6%, respectively. This could be due to higher temperatures in the Birni N'Gaouré, (southern region of Republic of Niger) area. Indeed, as reported by Rondanini *et al*. (2014), temperature during oil synthesis negatively affected the final oil content. Our findings are also in agreement with the results of Noorali *et al*. (2014), who studied several oils from various olive cultivars and found that all cultivars grown in Qom (a high temperature area) showed lower oil contents than those grown in Gorgan.

Results of the IV, SV, FFA, AV, PV, color and pH essays of GPKON and GPKOG are also shown in Table 1. GPKOG showed higher levels for all the oil indices when compared with GPKON.

Table 1. Physicochemical characteristic of gingerbread plum kernel oils

Parameter	GPKON*	GPKOG**
Oil content (% of dry weight basis)	56.15±1.77[b]	60.60±0.79[a]
Iodine value ($gI_2 \cdot 100\ g^{-1}$)	34.9±0.00[b]	39.12±1.83[a]
Saponification value ($mgKOH \cdot g^{-1}$)	153.34±0.32[b]	162.69±0.00[a]
Free fatty acid (% oleic acid)	0.33±0.02[a]	0.34±0.01[a]
Acid value ($mgKOH \cdot g^{-1}$)	0.67±0.03[a]	0.68±0.03[a]
Peroxide value ($meq\ O_2 \cdot kg^{-1}$)	41.28±0.06[b]	54.06±0.52[a]
Color		
*L**	91.47±0.01[a]	89.44±0.02[b]
*a**	−1.27±0.03[b]	−2.87±0.04[a]
*b**	69.42±0.02[b]	76.01±0.03[a]

Means of three determinations±SD; Mean values in rows with different letters were significantly different (Tukey's test); significance at ($p<0.05$) (analysis of variance).
*GPKON: Gingerbread plum kernel oil from Niger; **GPKOG: Gingerbread plum kernel oil from Guinea. L*=lightness (0=black; 100=white), a*=redness/greenness (+=red; −=green), b*=yellowness/blueness (+=yellow; −=blue).

3.1.2. Iodine Value

The iodine values were 34.9 and 39.12 g $I_2 \cdot 100g^{-1}$ for GPKON and GPKOG respectively (Table 1). These values are higher than the value of 32.07± 0.01 g $I_2 \cdot 100g^{-1}$ reported for gingerbread kernel oil from Nigeria (Warra, 2012). However, GPKON and GPKOG are more saturated than palm (IV 44–54), peanut (IV 82–107), corn (IV 103–128), cottonseed (IV 99–113) or linseed (IV 155–205) oils and considerably less saturated than coconut (IV 7.7–10.5) oil (Gunstone *et al*., 2002).

3.1.3. Saponification Value

SV of GPKON and GPKOG were recorded as 153.34 and 162.69 mg of mg $KOH \cdot g^{-1}$, respectively (Table 1). Unlike GPKOG, GPKON showed similar SV when compared with the value of 153.30 mg $KOH \cdot g^{-1}$ reported by Warra (2012). The SV in this study is lower than the values reported for seinat seed oil (186.20 mg $KOH \cdot g^{-1}$) (Azhari *et al*., 2014) and Nigerian cotton seed oil (199.42 mg $KOH \cdot g^{-1}$), but higher than those of castor seed oil (123.3 mg $KOH \cdot g^{-1}$) (Warra *et al*., 2011) and cashew kernel oil (137 mg $KOH \cdot g^{-1}$) (Akinhanmi *et al*., 2008). However, without any other analytical measurement, saponification values overlap too much to identify individual fats or oils.

3.1.4. Free Fatty Acid and Acid Value

FFA is one of the most frequently determined quality indices during the production, storage, and marketing of oils. GPKON and GPKOG showed a low level of FFA values, 0.33 and 0.34% oleic acid, respectively (Table 1) which are much low than the values for polyalthia longifera seed oil (7.73%) (Oyedeji *et al*., 2011), monodora myristica gaeth dunat (7.20%) (Ibironke, 2010) and rubber seed oil (7.55%) (Jumat and Bashar, 2009). Hydrolysis causes the triacylglycerol molecule to split at the ester linkage to form free fatty acids, di- and monoacylglycerols, and eventually free glycerol. The liberated free fatty acids have a distinct flavor and odor which are more disagreeable when the fatty acid chain length is shorter than 14 carbons. Thus, GPKON and GPKOG, which contain mostly C_{18} and C_{20} fatty acids (Table 2), may not become easily unpalatable until the FFA exceeds a certain level. The AV, which indicates the level of FFA as a result of lipase activity in oil, ranged from 0.67 to 0.68 mg KOH per g oil (Table 1).

Table 2. Fatty acid and tocopherol composition of gingerbread plum kernel oils

Fatty acid (%)	GPKON*	GPKOG**
Saturated (SFA)		
Palmitic acid (16:0)	9.18±0.02[a]	7.34±0.01[b]
Stearic acid (18:0)	5.21±0.33[b]	7.99±0.03[a]
Arachidic acid (20:0)	0.29±0.07[b]	0.41±0.01[a]
Lignoceric acid (24:0)	0.59±0.07[a]	0.61±0.01[a]
Behenic acid (22:0)	0.65±0.01[b]	0.83±0.07[a]
Mono unsaturated (MUFA)		
Oleic acid (18:1)	42.46±0.28[a]	41.43±0.01[b]
Eicosenoic acid (20:1)	0.49±0.07[a]	0.53±0.00[a]
Erucic acid (22:1n9)	5.37±0.03[a]	0.54±0.07[b]
Poly unsaturated (PUFA)		
Linoleic acid (18:2)	17.42±0.04[b]	18.67±0.03[a]
Arachidonic acid (20:4)	17.67±0.02[b]	21.72±0.00[a]
SFA	15.90±0.30[b]	17.18±0.05[a]
MUFA	48.31±0.30[a]	42.51±0.02[b]
PUFA	35.08±0.06[b]	40.40±0.01[a]
MUFA/PUFA	1.38±0.01[a]	1.050.00[b]
Tocopherols (%)		
α-Tocopherol	97.88±0.01[a]	62.96±0.00[b]
β-Tocopherol	2.06±0.01[b]	32.67±0.10[a]
γ-Tocopherol	ND	ND
δ-Tocopherol	0.06±0.01[b]	4.34±0.14[a]

Means of three determinations±SD; Mean values in rows with different letters were significantly different (Tukey's test); significance at ($p<0.05$) (analysis of variance).
*GPKON: Gingerbread plum kernel oil from Niger; **GPKOG: Gingerbread plum kernel oil from Guinea. ND=Not detected.

3.1.5. Peroxide Value

The oxidation of oils is a major cause of their deterioration. Hydroperoxides formed by the reaction between oxygen and unsaturated fatty acids are the primary products of this reaction. PV measures

the concentration of these substances and is often used as an indicator of oilseed quality related to oil oxidation. The PV in GPKON and GPKOG ranged from 41.28 to 54.06 mequiv O_2 per kg oil (Table 1). These values are higher than those in lam seed oil (20 meq·kg^{-1}) (Anhwange *et al.*, 2010) and stercula setegera seed oil (35.0 meq·kg^{-1}) (Kyari, 2008). Indeed, the solubility of oxygen in oil is about 3–5 times greater than in water. The amount of oxygen present in oil, dissolved during manipulation, is sufficient to oxidize the oil to a PV of around 10 (Przybylski and Eskin, 1988). Thus, the higher values found in this study may be due to improper manipulation and/or poor storage conditions.

3.1.6. Color Attributes

The color attribute (L*, a*, b*) values of GPKON and GPKOG were 91.47±0.01,−1.27±0.03, 69.42±0.02 and 89.44±0.02,−2.87±0.04, 76.01±0.03, respectively (Table 1). The investigated oils in this study were lighter when compared with other vegetable oils such as seinat seed oil (L*=64.6), palm, soybean, sunflower, olive and corn oils with L* values ranging from 63.4 to 69.5 (Hsu and Yu, 2002; Azhari *et al.*, 2014). This indicates that GPKON and GPKOG contain less yellow pigments like carotenoid compounds. On the other hand, GPKON with b value of 89.44 appears more yellow than GPKOG.

3.2. Fatty Acid Composition

The FA compositions of GPKON and GPKOG are presented in Table 2. The major monounsaturated fatty acid (MUFA) in both samples was oleic acid; 42.46% (GPKON) and 41.43% (GPKOG). Linoleic acid and arachidonic acid were the polyunsaturated fatty acids (PUFA) with arachidonic acid present in higher levels (17.67% and 21.72% in GPKON and GPKOG, respectively) than those in common vegetable oils. The main SFA were palmitic acid and stearic acid and the remaining fatty acids occurred in small amounts (<1%). The percentages of SFA, MUFA, and PUFA and the ratio of MUFA/PUFA for both samples were also investigated. Total SFA values were 15.90 and 17.18 for GPKON and GPKOG, respectively. These results are in the range of those (14.87 and 20.88%) reported by Noorali *et al.* (2014) for several olive cultivars grown in different areas, but lower than the values for coconut (86.5%) and plam oils (47.8%) (Foster *et al.*, 2009). The MUFA contents in GPKON and GPKOG; (48.31 and 42.51% respectively) (Table 2) were much higher than those reported for safflower (12.0%), sunflower (20.5%), soybean (21.3%), corn (29.9%) and palm (37.1%) oils (Zhang *et al.*, 2009). While PUFA values were found to be 35.08% (GPKON) and 40.40% (GPKOG) due to the high levels of linoleic and arachidonic acids found in this study. The MUFA/PUFA ratio is an important parameter for oil stability in highly unsaturated oils. Thus, regarding fatty acid composition, GPKON can be considered as a little bit more stable due to its relatively higher MUFA/PUFA ratio. However, the MUFA/PUFA values found in this study (1.38 for GPKON and 1.05 for GPKOG) are much lower than those reported by Moayedi *et al.* (2011) for several wild almond specie oils and are categorized as AZ (3.86), AH (3.24) and AJ (3.34).

3.3. Antioxidant Activity

Preliminary antioxidant tests revealed DPPH radical scavenging activity and reducing power in a dose–dependent manner (Fig. 2 and Fig. 3). The IC_{50} values of DPPH radical scavenging activity and $AC_{0.5}$ values for the reducing power of GPKON and GPKOG are shown in Table 3. Lower IC_{50} or $AC_{0.5}$ values indicate stronger antioxidant activities. The results of DPPH radical-scavenging activities and reducing power showed significant differences ($p<0.05$). GPKOG exhibited two times greater DPPH

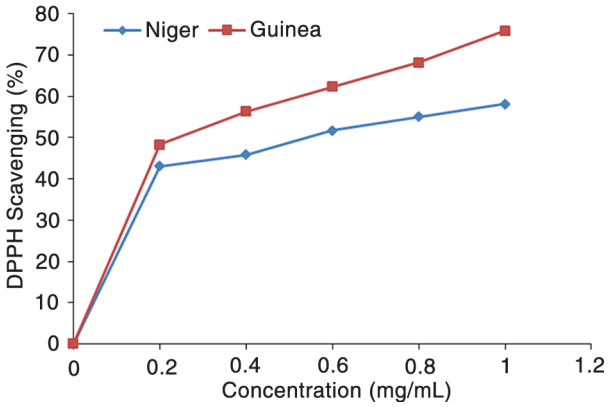

FIGURE 2. DPPH radical scavenging ability of gingerbread plum kernel oils at different concentrations.

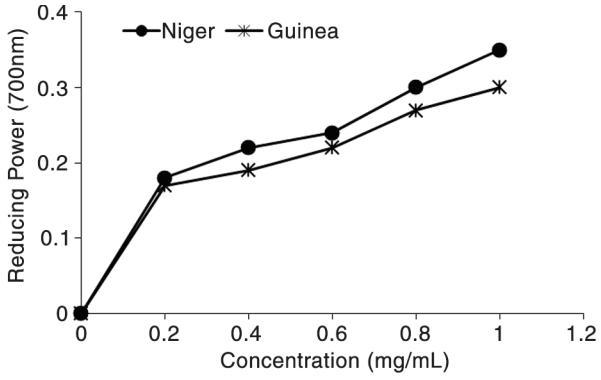

FIGURE 3. Reducing power of gingerbread plum kernel oils at different concentrations.

TABLE 3. Antioxidant activity of gingerbread plum kernel oils

Parameter	GPKON*	GPKOG**
DPPH IC_{50} ($mg \cdot mL^{-1}$)	0.56 ± 0.01^{b}	0.24 ± 0.01^{a}
Reducing power $AC_{0.5}$ ($mg \cdot mL^{-1}$)	1.76 ± 0.08^{a}	2.18 ± 0.06^{b}
Beta carotene ($mg \cdot mL^{-1}$)	1.53 ± 0.02^{b}	2.06 ± 0.01^{a}
Phenolic content (mg GAE·kg^{-1} sample)	184.22 ± 2.98^{b}	250.77 ± 1.13^{a}
Flavonoid ($\mu g \cdot mL^{-1}$)	86.25 ± 0.19^{a}	71.11 ± 0.13^{b}

Means of three determinations±SD; Mean values in rows with different letters were significantly different (Tukey's test); significance at ($p<0.05$) (analysis of variance).
*GPKON: Gingerbread plum kernel oil from Niger; **GPKOG: Gingerbread plum kernel oil from Guinea.

radical scavenging activity, (IC_{50}=0.24 $mg \cdot mL^{-1}$) than GPKON (IC_{50}=0.56 $mg \cdot mL^{-1}$) while GPKON had better reducing power with an $AC_{0.5}$ value of 1.76 $mg \cdot mL^{-1}$ (Table 3). On the other hand, GPKON and GPKOG showed higher DPPH radical scavenging activity when compared with different palm oils categorized as crude palm olein (IC_{50}=19.2 $mg \cdot mL^{-1}$), crude palm oil (IC_{50}=20.9 $mg \cdot mL^{-1}$) and crude palm stearin (IC_{50}=30.7 $mg \cdot mL^{-1}$) (Kumar and Krishna, 2014). As for β-carotene and flavonoid contents, the values were found to be 1.53; 2.06 $mg \cdot mL^{-1}$ and 86.25; 71.11 $\mu g \cdot mL^{-1}$ for GPKON and GPKOG, respectively (Table 3). It is well-documented that β–carotene and flavonoids are natural antioxidants. Flavonoids can directly react with superoxide anions and lipid peroxyl radicals and consequently inhibit or break the chain of lipid peroxidation. In the case of β-carotene, some authors reported values of 6.43 $mg \cdot kg^{-1}$ and 5 $mg \cdot kg^{-1}$ for extra virgin olive oil (Anniva and Tsimidou, 2009).

3.4. Total Phenolic Content

The total phenol contents of GPKON and GPKOG are expressed as their equivalent gallic acid in mg per kg oil. The values are 184.22 $mg \cdot kg^{-1}$ (GPKON) and 250.77 $mg \cdot kg^{-1}$ (GPKOG) (Table 3). The value for regular almond was 37.7 $mg \cdot kg^{-1}$ (Moayedi *et al*., 2011), which is much more inferior to the values found in this study. Wijeratne *et al*. (2006) reported a total phenolic content of 8 mg (quercetin equiv·g^{-1} of ethanolic extract) in whole almond seed while Laincer *et al*. (2014) found a level of 218.66 $mg \cdot kg^{-1}$ total phenolic contents present in olive oils from Algeria. Our results are in the range of these findings. A direct relationship has been found between the contents of total phenolics and the antioxidant capacity of plants. Thus, GPKOG, presenting the higher level of total phenolic content, appeared also to be more active as an antioxidant source.

3.5. Tocopherol Composition

The tocols (tocopherols and tocotrionols) are the most important class of antioxidants naturally present in vegetables oils and fats. These natural fat-soluble antioxidants exist in at least seven forms with α, β, γ, and δ predominating in most vegetable oils. Data about the qualitative composition of tocopherols are summarized in Table 2 and Fig. 4. Three tocopherols were found, wherein α-tocopherol constituted 97.88 and 62.96% of the total in GPKON and GPKOG, respectively, followed by β-tocopherol and δ-tocopherols in both samples (Table 2). ϒ-tocopherol and α-tocopherol proved to be the main tocopherols in vegetable oils and fats (Schwartz *et al*., 2008) even though γ-tocopherol was not detected in the present study. ϒ-tocopherol is the major tocopherol present in soybean oil with the sigma, alpha and beta compounds present in decreasing quantities; 62.8, 26.7, 9.3 and 1.2%, respectively (Gunstone *et al*., 2002). Values of 42.5% β-tocopherol, 41.3% γ-tocopherol, 15.9% α-tocopherol and 0.3% δ-tocopherol were found in canterbury bell (Campanula medium) seed oil (Hassaniena *et al*., 2014). The antioxidant properties of tocopherols may be involved in combating atherosclerosis by preventing the oxidation of low-density lipoproteins. The levels of tocopherols detected in gingerbread plum kernel may contribute to the stability of GPKON and GPKOG toward oxidation.

3.6. Sterol composition

The sterol composition of GPKON and GPKOG is shown in Table 4. Eleven sterol compounds were detected in both samples, wherein 24-hydroxy-24-methyl cholesterol, sitosterol and clerosterol accounted for 68.5% and 66.33% in GPKON and GPKOG respectively. The sterol marker was 24-hydroxy-24-methyl cholesterol (39.34% in GPKON and 41.55% in GPKOG) followed by sitosterol and clerosterol. Other components such as 7-α-hydroxycholesterol, Δ^5-avenasterol, 24-dihydrolanosterol, sitostanol, β-amyrin, 24-hydroxycholesterol, campesterol and phytol were found in low levels (<7%), (Table 4). Among the different phytosterols, β-sitosterol has been most intensively investigated with respect to its beneficial and physiological effects on health. β-sitosterol lowers cholesterol levels, enhances immunity, and has anti-inflammatory, antipyretic and anti-carcinogenic effects (prostate essentially)

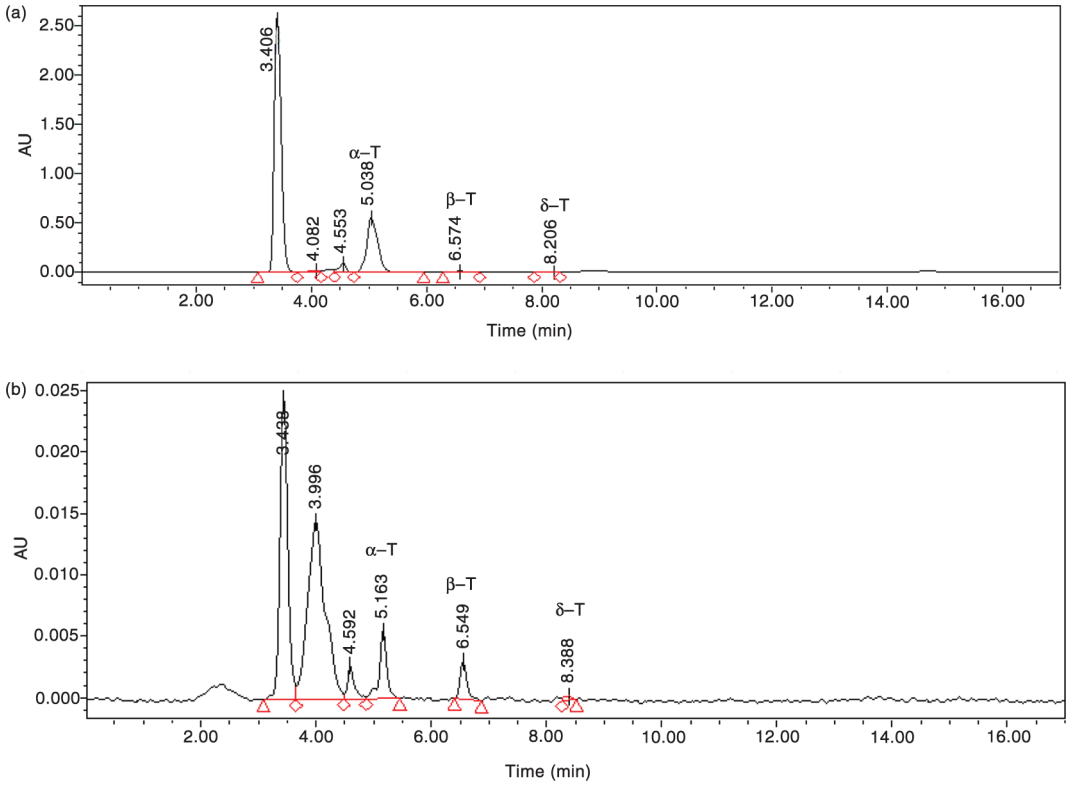

FIGURE 4. Tocopherol HPLC-chromatogram of gingerbread plum kernel oils (a: oil from Niger; b: oil from Guinea).

(Pegel, 1997; Kritchevsky and Shirley, 2005). However, compared with other vegetable oils, GPKON and GPKOG contain less sitosterol; 16.18% and 13.17%, respectively. Reported values of β-sitosterol were 88% and 91% in wild pistachio and wild almond seed oils respectively (Givianrad *et al.*, 2013), 54.1% in soybean, 60.3% in corn, and 46.1% in coconut oils (Gunstone *et al.*, 2002) while, Δ^5-avenasterol was found to be 2.5%, 10.5% and 27.4% in soybean, corn and coconut oils respectively and campesterol accounted for 18.1%, 17.2% and 8.8% in soybean, corn and coconut oils (Gunstone *et al.*, 2002). The values for Δ^5-avenasterol and campesterol in GPKON and GPKOG were 4.47%, 6.73% and 4.82, 2.36%, respectively (Table 4). Since the structure of phytosterols resembles that of cholesterol, these compounds may be involved in similar oxidative reactions. Przybylski and Eskin (1988) found some oxidation products formed from plant sterols during the storage of fried food products. However, as sterols are affected by processing; about 40% of these components can be removed from the oil during deodorization.

TABLE 4. Sterol composition of gingerbread plum kernel oils (%)

Parameter	GPKON*	GPKOG**
7-α-hydroxycholesterol	3.6±0.21^b	7.5±0.21^a
24-hydroxy-24-methyl cholesterol	39.34±0.42^b	41.55±0.18^a
Δ^5-avenasterol	4.47±0.13^b	6.73±0.00^a
24-dihydrolanosterol	4.82±0.04^a	3.55±0.02^b
Sitostanol	3.67±0.01^a	2.83±0.07^b
β-amyrin	1.3±0.06^b	1.72±0.22^a
24-hydroxycholesterol	6.74±0.01^a	4.49±0.03^b
Campesterol	4.82±0.01^a	2.36±0.01^b
Clerosterol	12.78±0.04^a	11.61±0.23^b
Sitosterol	16.18±0.07^a	13.17±0.08^b
Phytol	2.3±0.07^b	4.51±0.01^a

Means of three determinations±SD; Mean values in rows with different letters were significantly different (Tukey's test); significance at ($p<0.05$) (analysis of variance).
*GPKON: Gingerbread plum kernel oil from Niger; **GPKOG: Gingerbread plum kernel oil from Guinea.

4. CONCLUSIONS

In spite of their wide distribution and high nutritional value, gingerbread plum kernels have not yet been used for industrial applications. Indeed, it is difficult to find data about the bioactive lipids and antioxidant characteristics of gingerbread plum kernel oil in the literature and as far as we know, this is the first detailed report concerning gingerbread plum kernel oils. Analyses of the oils extracted from

gingerbread plum kernels grown in two different areas (Birni N'Gaouré, southern region of Republic of Niger and Gaoul in the Boke region, Republic of Guinea) showed that they have different characteristics. So, improved knowledge about gingerbread plum kernel oils would assist in efforts to achieve industrial applications for these underutilized seeds.

ACKNOWLEDGMENTS

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

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