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EFFECTS OF FRUIT MATURITY ON OXIDATIVE STABILITY OF *LAGENARIA SICERARIA* (MOLINA) STANDL. SEED OIL EXTRACTED WITH HEXANE

AHOU LETICIA LOUKOU[1,5], GEORGES LOGNAY[2], JEAN-PIERRE BAUDOIN[3], LUCIEN PATRICE KOUAME[1] and BI IRIE ARSENE ZORO[4]

[1]Unité de Formation et de Recherche des Sciences et Technologies des Aliments, Université d'Abobo-Adjamé, 02 BP 801 Abidjan 02, Côte d'Ivoire
[2]Unité de Chimie Analytique, Gembloux Agro-Bio Tech (GxABT), Université de Liège, Belgium
[3]Unité de Phytotechnie Tropicale et Horticulture, Gembloux Agro-Bio Tech (GxABT), Université de Liège, Belgium
[4]Unité de Formation et de Recherche des Sciences de la nature, Université d'Abobo-Adjamé, Côte d'Ivoire

[5]Corresponding author. TEL: +225-02979081; EMAIL: loukouletine@yahoo.fr

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ABSTRACT

The effects of fruit maturity on oxidative stability of LSO were investigated using an accelerated test at 60C for 6 days. Fatty acid composition, Cox, PV, *p*-AV, specific extinction at 232 and 268 nm, and TOTOX were used to assess LSO stability. LSO samples from berry seeds at 50-DAFS, at CPW and at CPW + S60 were analyzed. LSO at CPW was significantly more stable to oxidation than LSO at 50-DAFS and CPW + S60. This oil had lower *p*-AV than control (peanut oil) and other sources of LSO. PV, specific extinction, TOTOX and Cox of LSO at CPW were also lower than those of 50-DAFS and CPW + S60, but two times higher than control values. Collectively, these data suggest that LSO cannot be used after 3 days of storage at 60C.

PRACTICAL APPLICATIONS

The results obtained showed that the seed oil of oleaginous gourd *Lagenaria siceraria* (Molina) Standl. was a good source of PUFAs (linoleic acid, an essential fatty acid) for human diet. LSO of CPW could be potentially used for cooking, seasonings and margarine applications because of its high nutritional value and its low linolenic acid content. These seeds could also be used by the food industry for formulating functional foods enriched with PUFAs.

INTRODUCTION

Cucurbits are one of the most economically important crops worldwide. They are cultivated for seed consumption, which are reported to be rich in nutrients (Achu *et al.* 2005). They are also well adapted to extremely divergent agro-ecosystems and various cropping systems characterized by minimal human input (El Tahir and Yousif 2004). They are grown in both temperate and tropical regions (Sanjur *et al.* 2002). *Lagenaria siceraria* (Molina) Standl. belongs to this category of crops and is one of the most widely distributed and consumed products in both rural and urban areas in sub-Saharan Africa. *L. siceraria* is the most widely cultivated oleaginous cucurbit for its high agronomic potential (Achigan Dako *et al.* 2006). It exhibits the richest macronutrient contents and contains 34% proteins and 50% fat (Achu *et al.* 2005). Fats and oils are very important in human diet. LSO is not produced traditionally in Côte d'Ivoire unlike palm oil, palm kernel oil and shea (*Butyrospermum paradoxum*) and makore (*Tieghemella heckelii*) butters because its consumption is not widespread. However, according to Morimoto and Mvere (2004), this oil is edible and sold in certain local markets of West African countries while only being consumed when other vegetable oils are scarce in South Africa despite its high nutritional content (56.41–66.70%) of PUFAs.

The high content of essential fatty acids in this crop contributes to the appropriate development of human tissues (Moya Moreno *et al.* 1999). LSO contains a large amount of PUFAs such as linoleic and linolenic acids, which are known to reduce the risk of atherosclerosis and cardiovascular

diseases (Hegsted *et al*. 1965; Abramovic *et al*. 2007). It has been claimed that *L. siceraria* seed oil has several beneficial health effects (Milind and Satbir 2011).

It is generally known that fats and oils can be deteriorated during storage in an oxidizing atmosphere, designated lipid oxidation. Lipid oxidation is probably the most important factor affecting the shelf life of edible oils. The hydroperoxides produced by lipid oxidation can be decomposed into various smaller molecules such as aldehydes, ketones, alcohols and carboxylic acids. Some of these volatile products influence flavor, even at very low concentrations resulting in oil and the food being rancid and unpalatable (Richardsa *et al*. 2005). This process does not only produce rancid flavor but also lowers the nutritional value of foods because of the formation of oxidation products, which may play a role in the development of disease and can be harmful to humans (Muik *et al*. 2005). Therefore, the evaluation of oxidative stability is a key factor in developing new oils for food applications.

The oil stability during storage or upon heating is a vital parameter for ensuring good performance at elevated temperatures. Oxidation of unsaturated fatty acids is one of the major causes of off-flavor compounds and in the reduction of nutritional value of food products (Hemalatha 2007). Minor components such as tocopherol, tocotrienols and polyphenols greatly affect oxidative stability (Bozan and Temelli 2008). The oxidation process mainly involves the degradation of PUFA and the generation of free radicals (Spiteller 2001). It has been shown that exposure to air, heat, light, trace metals and moisture enhances their chemical reactivity (Naz *et al*. 2004, 2005). It is therefore very important to determine the changes in fatty acid composition during maturation stages (Sebei *et al*. 2007; Seyhan *et al*. 2007). Belitz *et al*. (2004) reported a comprehensive description of the changes in acyl lipids of food following auto-, photo- and enzymatic alterations.

In the African traditional farming system (multiple cropping system on small fields), the oleaginous cucurbits are harvested at the complete wilting of the plants (Ban *et al*. 2006). The harvested fruits are often stored on farms for an undetermined period before seed extraction. Such cultural practices do not optimize the yield quality in terms of seed nutrients. Indeed, Rondanini *et al*. (2007) demonstrated that in several nonperennial crops, fruits and seeds can reach the morphological and physiological maturity before the senescence of plants. Physiological maturity occurs when chemicals reach their highest quantitative and qualitative levels in the fruits or seeds (Berti and Johnson 2008). There are numerous analytical methods to estimate deterioration of edible oil, such as Cox value (Farhoosh *et al*. 2009), PV, *p*-AV (Naz *et al*. 2004; Farhoosh *et al*. 2009), specific extinctions, and TOTOX value (Abramovic *et al*. 2007).

Despite the great nutritional value of the oleaginous gourd LSO, no information is available on its oxidative stability. To fill this knowledge gap, this study was conducted to evaluate the oxidative stability of the oleaginous gourd LSO at three stages of maturity.

MATERIALS AND METHODS

Samples and Chemicals

The seed oil of the oleaginous gourd *L. siceraria* (Molina) Standl was used in the present study. These seeds were extracted from fruits harvested at three different stages of maturation: 50-DAFS, CPW and CPW + S60. The seeds were obtained from the cucurbit germplasm collection of the University of Abobo-Adjame (Abidjan, Côte d'Ivoire). Commercial oil of peanut (*Arachis hypogaea*) was produced by Vandemoortele (Izegem, Belgium) and used as control. *p*-Anisidine was purchased from Sigma-Aldrich (St. Louis, MO). Isooctane and acetic acid were of spectrophotometric grade; all other solvents and reagents used were of analytical grade (VWR International, Leuven, Belgium).

Lipid Extraction and Preservation

A sample of crushed seeds (15 g) was introduced into a glass vessel and 50 mL of *n*-hexane was added. The whole mixture was stirred at room temperature and vacuum filtered. The residue was washed with 50 mL of solvent three times and then filtered. The filtrates were pooled and then filtered again. The solvent was evaporated in a rotary evaporator at 35C. Residues of solvent were then evaporated under a gentle stream of nitrogen. To maintain their freshness, the commercial peanut oil as well as those from the seed of *L. siceraria* were stored frozen at −20C until use.

Storage Conditions

Three samples (3 ± 0.1 g of oil for each treatment) were placed in a separate 25-mL open glass flak (30 mm in diameter) and stored in a dark oven (Memmert ICP800 incubator, Schwabach, Germany) at 60 ± 0.5C for up to 0–6 days. After each storage period, oil samples were immediately analyzed.

Analysis of Fatty Acids

The fatty acids extracted from the LSO were analyzed using gas chromatography (Hewlett–Packard 6890, Agilent Technologies, Brussels, Belgium) with a flame ionization detector at 265C. The gas carrier was helium and the pressure was maintained at 128.9 kPa. For analytical purposes, 1.0 μL of each sample was injected at a flow rate of 1.8 mL/min (column Varian CP 9205, Sint-Katelijne-Waver, Belgium, 30 m length, 0.25 mm diameter and 0.25 μm thickness of film). Esterification of the fatty acids was performed using the

method of Morrisson and Smith (1964). According to this method, 10 mg of LSO was converted to fatty acid methyl esters (FAMEs) using boron trifluoride methanol (14% w/v). After dissolution in hexane, 1.0 µL of extract containing FAMEs was injected into the column of GC. Peaks were integrated by a Shimadzu C-R3A computing integrator, which was calibrated with a reference methyl ester fatty acid obtained from Supelco Inc. (N 47119; Bellefonte, PA). The degree of unsaturation was calculated by addition of the unsaturated fatty acids. The identification and quantification of FAMEs were accomplished by comparing the retention times and peak areas of the standard FAMEs. The identification was confirmed by gas chromatography–mass spectrometry.

Evaluation of Oxidative Stability

The oxidative stability of LSO was evaluated by determining the fatty acid composition by GC and measuring the FAMEs, Cox, specific extinction at 232 and 268 nm, *p*-AV, PV and TOTOX.

Cox value of the oils was based on the percentage of unsaturated C18 fatty acids, as described by (Fatemi and Hammond 1980):

$$\text{Cox} = (1[\text{C18:1\%}] + 10.3[\text{C18:2\%}] + 21.6[\text{C18:3\%}])/100 \quad (1)$$

where C18:1, C18:2 and C18:3 are oleic, linoleic and linolenic acids, respectively.

Lipid oxidation was determined by specific extinction values at 232 and 268 nm with Ch 5–91 method of AOCS (1997) using UV-1800 spectrophotometer (Shimadzu, Tokyo, Japan). Oil samples were diluted in isooctane (1%), the absorbances obtained were used for calculating the specific extinction ($E^{1\%}_{1\,\text{cm}}$) as follows:

$$E^{1\%}_{1\,\text{cm}} = A_\lambda/(C \times 1) \quad (2)$$

where $E^{1\%}_{1\,\text{cm}}$ is the specific extinction, A_λ is the absorbance measured at either 232 nm (K_{232}) or 268 nm (K_{268}), C is the concentration of the oil solution in g/100 mL, and *1* represents the path length of the cuvette in cm.

The method Cd 8b-90 (AOCS 1997) was used to determine PVs. LSO sample (0.5 g) was dissolved in 10 mL of acetic acid : isooctane (3:2, v/v) and 50 µL of potassium iodide saturated solution was added. The mixture was shaken for 1 min, 3 mL of distilled water was added, and the mixture was titrated with sodium thiosulfate (0.01 N) until disappearance of the yellow color. Then, 50 µL of sodium dodecyl sulfate (10%) and 50 µL of starch indicator (1 g in 200 mL boiling distilled water) were added. Titration was continued until the yellow color just disappeared. The blank was also analyzed under similar conditions. PV (meq/kg) was calculated according to the equation: $\text{PV} = N \times (V - V_K) \times 1{,}000/m$, where N is the normality of sodium thiosulfate (N), V and V_k are the volume (mL) of sodium thiosulfate exhausted by sample and blank, respectively, and m is the mass of LSO (g).

To determine *p*-AVs by Cd 18–90 method (AOCS 1997), oil samples (0.5 g) were dissolved in 10 mL of isooctane. One milliliter of *p*-anisidine (0.25%) in acetic acid (w/v) was added to 5 mL of the above mixture, and after 10 min, the absorbance was read at 350 nm using a spectrophotometer (UV-1800, Shimadzu). A blank made with isooctane and *p*-anisidine solution was realized. *p*-AV was calculated according to the equation:

$p\text{-AV} = 10 \times (1.2[A_s - B] - A_b)/m$, where A_s is the absorbance of the fat solution after reaction with the *p*-anisidine reagent, B is the blank absorbance, A_b is the absorbance of the fat solution and m is the mass of LSO (g) sample.

TOTOX value was calculated as follows:

$$\text{TOTOX} = 2(\text{PV}) + p\text{-AV} \quad (3)$$

Statistical Analysis

All experiments about fatty acid composition were performed on four replicate samples. Other analyses were conducted in triplicate. For each examined parameter, an analysis of variance (ANOVA) was performed using the SAS statistical package (SAS 1999). ANOVA was used to determine the effect of maturity stage on the percentage of fatty acids and on the oxidative stability. When a significant difference was observed between the stages of maturity for a parameter, the ANOVA was supplemented by least significant difference (LSD) multiple range tests. This test was used to identify differences among the means of parameters examined. Student's *t*-test was used to compare the LSO stability to that of peanut.

RESULTS

Fatty Acid Composition of Oils

The fatty acid compositions of *L. siceraria*, palm, olive and peanut oils are listed in Table 1. The statistical analysis showed a significant effect of the stages of maturity on the eight fatty acids, the PUFA/SFA ratio and Cox value of LSOs. The saturated fatty acids comprising of palmitic, stearic, arachidic and lignoceric acids were highest in oils from seeds of berries harvested at CPW + S60. MUFAs (palmitoleic and oleic acids) and PUFAs (linoleic and linolenic) were, respectively, highest in oils from seeds of berries harvested at CPW and 50-DAFS. The PUFA/SFA ratio and Cox value of oil at 50-DAFS were significantly higher than those of the oil at CPW and at CPW + S60.

The percentage of SFAs (palmitic acid [C16:0], arachidic acid [C20:0], stearic acid [C18:0] and lignoceric acid [C24:0])

TABLE 1. FATTY ACID COMPOSITION OF *LAGENARIA SICERARIA* SEED OILS FROM BERRIES AT DIFFERENT STAGES OF MATURITY AND AVERAGE VALUES FOR SOME COMMERCIAL OILS

				Maturity stages of *Lagenaria siceraria* (*n*† = 4)				
Fatty acids (%)‡	Palm oil*	Olive oil*	Peanut oil	50-DAFS	CPW	CPW + S60	*F*	*P*
Lauric (C12:0)	0.10	nd	nd	nd	nd	nd	–	–
Myristic (C14:0)	1.00	nd	nd	nd	nd	nd	–	–
Palmitic (C16:0)	43.00	10.2	07.90 ± 0.04	13.85 ± 0.04^{b}	13.55 ± 0.03^{c}	14.38 ± 0.04^{a}	543.49	≤0.001
Margaric (C17:0)	nd	<0.10	nd	nd	nd	nd	–	–
Stearic (C18:0)	5.00	3.15	01.90 ± 0.02	06.96 ± 0.01^{a}	06.31 ± 0.01^{c}	06.86 ± 0.01^{b}	3,827.15	≤0.001
Arachidic (C20:0)	0.40	0.42	02.90 ± 0.02	00.29 ± 0.01^{a}	00.28 ± 0.01^{ab}	00.27 ± 0.01^{b}	5.25	0.030
Behenic (C22:0)	nd	0.11	00.20 ± 0.01	nd	nd	nd	–	–
Lignocéric (C24:0)	nd	<0.10	01.66 ± 0.01	00.09 ± 0.00^{c}	00.14 ± 0.00^{a}	00.10 ± 0.00^{b}	24.3	≤0.001
SFAs	49.50	13.88	14.55 ± 0.09	21.14 ± 0.09^{b}	20.24 ± 0.03^{c}	21.56 ± 0.05^{a}	516.18	≤0.001
Palmitoleic (C16:1)	0.10	0.75	00.05 ± 0.01	00.05 ± 0.00^{b}	00.06 ± 0.01^{a}	00.07 ± 0.00^{a}	12.57	0.019
Margaroleic (C17:1)	nd	0.10	nd	nd	nd	nd	–	–
Oleic (C18:1n9)	39.00	80.70	56.14 ± 0.12	12.05 ± 0.01^{c}	20.91 ± 0.05^{a}	16.34 ± 0.03^{b}	80,402.0	≤0.001
Gadoleic (C20:1)	nd	0.23	nd	nd	nd	nd	–	–
MUFAs	39.10	81.78	56.14 ± 0.12	12.07 ± 0.04^{c}	20.96 ± 0.03^{a}	16.37 ± 0.04^{b}	61,972.7	≤0.001
Linoleic (C18:2n6)	11.00	3.69	26.06 ± 0.05	66.63 ± 0.12^{a}	58.62 ± 0.05^{c}	62.05 ± 0.13^{b}	5,729.91	≤0.001
Linolenic (C18:3n3)	0.20	0.61	00.23 ± 0.00	00.15 ± 0.00^{a}	00.10 ± 0.01^{b}	00.09 ± 0.00^{c}	513.00	≤0.001
PUFAs	11.20	4.30	26.29 ± 0.1	66.78 ± 0.12^{a}	58.72 ± 0.05^{c}	62.14 ± 0.13^{b}	5,748.60	≤0.001
PUFAs/SFAs	0.23	0.31	01.80 ± 0.01	03.17 ± 0.00^{a}	02.92 ± 0.00^{b}	02.89 ± 0.00^{c}	15,933.3	≤0.001
Cox value	1.57	1.32	03.29 ± 0.01	07.02 ± 0.01^{a}	06.27 ± 0.01^{c}	06.57 ± 0.01^{b}	4,483.01	≤0.001

* Source: Young 1987 (palm oil) and Pardo *et al*. 2007 (olive oil).

† Sample size (n = 4); standard deviation is given after ±; in line.

‡ For each fatty acid, mean values followed by the different superscript letters were significantly different based on least significant difference test at 0.05 level.

nd, nondetected.

in olive and peanut oil were 1.5 times lower than the corresponding values of *L. siceraria* seed oils. There were two predominant fatty acids in palm oil, C16:0 (43.00%) and C18:1 (39.00%), with a percentage of SFAs approximately 2.3 times higher than those of *L. siceraria*. The percentage of MUFAs in peanut oil was 4.65, 2.68 and 3.43 times higher than those in the LSO at 50-DAFS, CPW and CPW + S60, respectively. Olive oils showed a preponderance of MUFAs (81.78%). The percentage of PUFAs in peanut oil was less than half of that in LSOs.

The PUFA/SFA ratio and Cox value of peanut oil represented half of those in *L. siceraria* seed oils. On the other hand, Cox value and PUFA/SFA ratio of olive and palm oils were, respectively, 5 and 10 times lower than those of LSOs.

Oxidative Stability of *L. siceraria* Seed Oils

The peroxide values of LSO during storage at 60C are shown in Table 2. The general trend indicated a significant effect of the maturity stages. Oil at CPW presented the lowest values of peroxide, while the highest value was obtained at CPW + S60.

The changes in specific extinction at 232 nm from day 0 to day 6 in *L. siceraria* seed oils from fruits harvested at 50-DAFS, CPW and CPW + S60 are shown in Table 2. K_{232} values of *L. siceraria* seed oils varied significantly among the fruit maturity stages. K_{232} from day 0 to day 1 for oil at CPW + S60 was lower than those of the other oils. After day 1, K_{232} of oils at CPW became the lowest.

The *p*-AV provides information about secondary oxidation product, like aldehydes. The *p*-AVs for the different oils examined are shown in Table 3. No significant increase ($P \geq 0.05$) in *p*-AV was observed for oil at CPW. *P*-AV in oil at 50-DAFS decreased, while they increased in oil at CPW + SE60. The *p*-AV of oil at CPW was consistently lower, at all times, than those of the other oils.

The changes in specific extinction at 268 nm of the three oil categories are presented in Table 3. The results revealed no significant difference during the storage, except for oil at CPW + S60. The specific extinctions at 268 nm increased during the first 3 days and then decreased. Oil at CPW achieved lower K_{268} than the other stages.

TOTOX values are shown in Table 3. Increasing TOTOX values were observed for all oils during storage at 60C. TOTOX values with significantly low values were obtained at CPW. Those with significantly high and intermediary values were obtained at CPW + S60 and at 50-DAFS, respectively.

TABLE 2. CHANGES IN PV AND SPECIFIC EXTINCTION AT 232 nm (K_{232}) IN LSO FROM BERRIES SEEDS AT THREE MATURITY STAGES DURING 6 DAYS OF STORAGE AT 60C

		Maturity stages of *Lagenaria siceraria*‡ (*n** = 3)				
Parameters†	Day	50-DAFS	CPW	CPW + S60	*F*	*P*
PV (meq O_2/kg)	0	03.48 ± 0.09^{aG}	01.56 ± 0.00^{cG}	02.05 ± 0.12^{bG}	370.86	≤0.001
	1	05.62 ± 0.25^{bF}	03.67 ± 0.27^{cF}	06.46 ± 0.04^{aF}	134.23	≤0.001
	2	13.41 ± 0.07^{bE}	09.59 ± 0.11^{cE}	14.30 ± 0.04^{aE}	3,009.92	≤0.001
	3	21.21 ± 0.11^{bD}	15.52 ± 0.08^{cD}	22.13 ± 0.03^{aD}	5,673.24	≤0.001
	4	30.63 ± 0.28^{bC}	25.59 ± 0.03^{cC}	32.13 ± 0.06^{aC}	1,233.12	≤0.001
	5	40.05 ± 0.51^{bB}	35.66 ± 0.03^{cB}	42.14 ± 0.09^{aB}	372.02	≤0.001
	6	49.47 ± 0.73^{bA}	45.72 ± 0.08^{cA}	52.14 ± 0.12^{aA}	167.81	≤0.001
F		6,632.12	58,106.1	162,974		
P		≤0.001	≤0.001	≤0.001		
K_{232}	0	2.90 ± 0.07^{aF}	2.73 ± 0.06^{aF}	2.30 ± 0.38^{bF}	10.44	0.011
	1	2.57 ± 0.00^{bF}	2.82 ± 0.07^{aF}	2.58 ± 0.15^{bF}	6.83	0.028
	2	3.57 ± 0.17^{bE}	3.76 ± 0.12^{bE}	5.01 ± 0.09^{aE}	105.57	≤0.001
	3	4.57 ± 0.34^{bD}	4.70 ± 0.31^{bD}	7.43 ± 0.25^{aD}	85.22	≤0.001
	4	6.03 ± 0.27^{bC}	5.94 ± 0.28^{bC}	7.98 ± 0.16^{aC}	66.95	≤0.001
	5	7.49 ± 0.21^{bB}	7.18 ± 0.26^{bB}	8.54 ± 0.07^{aB}	39.75	≤0.001
	6	8.95 ± 0.15^{aA}	8.41 ± 0.24^{bA}	9.09 ± 0.04^{aA}	13.95	0.005
F		430.42	309.87	834.65		
P		≤0.001	≤0.001	≤0.001		

* Sample size (n = 3); standard deviation is given after ±; in line.

† For each parameter, mean values followed by the different superscript letters (a–c) were significantly different based on least significant difference (LSD) test at 0.05 level.

‡ For each maturity stage, mean values followed by the same superscript letters (A–G) were significantly different based on LSD test at 0.05 level.

Comparison of Oxidative Stability of *L. siceraria* and *Arachis hypogaea* Oils

The peroxide values of *L. siceraria* and *A. hypogaea* oils during storage at 60C are reported in Fig. 1. These values increased gradually, with peanut oil producing the lowest peroxide values.

Figure 1 also showed the changes in the conjugated diene contents in *L. siceraria* and *A. hypogaea* oils during storage at 60C. Conjugated diene contents of the two oils increased gradually ($P < 0.05$) as the storage time increased. The lowest values of K_{232} were observed in the peanut oil.

The *p*-AVs of the secondary oxidation products at 60C (Fig. 2) showed no significant increase during the storage of LSOs at CPW and *A. hypogaea* oil. The *p*-AV showed the lowest values in LSO at CPW.

Figure 2 shows the variations of K_{268} in oil from seeds at CPW and peanut oil. Only specific extinctions at 268 nm of peanut oil declined and remained steady after 24 h of storage. Peanut oil had the lowest values of K_{268}.

TOTOX of *L. siceraria* and peanut oil increased during storage. In the initial oil of *L. siceraria*, TOTOX was lower than that of peanut. Figure 2 revealed that after day 0, TOTOX of peanut oil became the lowest.

DISCUSSION

Fatty Acid Composition of Oils

Oils are very important in human diet because of the high contents of essential fatty acids, which are necessary for the appropriate development of human tissues (Moya Moreno *et al*. 1999).

The fatty acid composition of LSO showed that it had a reasonably high content of unsaturated fatty acids, especially linoleic acid (58.62–66.63%) compared with SFAs. The high degree of unsaturation led to the low resistance to oxidative rancidity (Onyeike and Acheru 2002). LSO used in this study had a good initial quality. It contained high level of essential fatty acids and could be used for cooking and seasonings due to its low content in linolenic acid (<2%) as recommended (Fenart 2004). Based on the linoleic and linolenic acid contents, LSO from CPW and CPW + S60 should have a better oxidative stability than oils from seeds at 50-DAFS. It is noteworthy that the oxidative rates of oleic acid, linoleic acid and linolenic acid are in the ratio of 1:12:25 (Min and Boff 2002). The low PUFA/SFA ratio and Cox value of LSO of CPW and CPW + S60 means that oils at those stages would be effectively less susceptible to oxidation than LSO of 50-DAFS.

TABLE 3. CHANGES IN *p*-AV, SPECIFIC EXTINCTION AT 268 nm (K268 nm) AND TOTOX IN LSO FROM BERRIES SEEDS AT THREE MATURITY STAGES DURING 6 DAYS OF STORAGE AT 60C

		Maturity stages of *Lagenaria siceraria*‡ (*n** = 3)				
Parameters†	Day	50-DAFS	CPW	CPW + S60	*F*	*P*
p-AV	0	2.75 ± 0.03^{aA}	0.93 ± 0.02^{b}	1.00 ± 0.05^{bF}	1,679.71	≤0.001
	1	0.88 ± 0.40^{B}	0.93 ± 0.01	1.39 ± 0.21^{E}	3.45	0.101
	2	0.96 ± 0.19^{bB}	0.90 ± 0.02^{b}	1.59 ± 0.12^{aD}	25.57	≤0.001
	3	1.05 ± 0.05^{bB}	0.87 ± 0.04^{c}	1.79 ± 0.03^{aC}	408.74	≤0.001
	4	1.07 ± 0.03^{bB}	0.89 ± 0.03^{c}	1.88 ± 0.02^{aBC}	1,228.69	≤0.001
	5	1.09 ± 0.02^{bB}	0.91 ± 0.02^{c}	1.96 ± 0.01^{aAB}	3,243.44	≤0.001
	6	1.11 ± 0.04^{bB}	0.92 ± 0.02^{b}	2.05 ± 0.02^{aA}	1,841.95	≤0.001
F		29.42	2.30	45.58		
P		≤0.001	0.093	≤0.001		
K_{268}	0	2.86 ± 0.07^{ab}	2.50 ± 0.05^{b}	3.26 ± 0.38^{aE}	8.80	0.016
	1	2.76 ± 0.04^{b}	2.61 ± 0.06^{b}	3.58 ± 0.21^{aD}	50.86	≤0.001
	2	2.80 ± 0.12^{b}	2.68 ± 0.06^{b}	4.55 ± 0.06^{aC}	435.62	≤0.001
	3	2.84 ± 0.21^{b}	2.76 ± 0.18^{b}	5.52 ± 0.09^{aA}	259.88	≤0.001
	4	2.85 ± 0.16^{b}	2.71 ± 0.14^{b}	4.90 ± 0.06^{aB}	287.80	≤0.001
	5	2.86 ± 0.10^{b}	2.66 ± 0.01^{c}	4.28 ± 0.03^{aC}	342.82	≤0.001
	6	2.87 ± 0.05^{b}	2.62 ± 0.06^{c}	3.66 ± 0.00^{aD}	456.93	≤0.001
F		0.32	1.97	67.73		
P		0.913	0.139	≤0.001		
TOTOX	0	09.61 ± 0.02^{aG}	04.04 ± 0.02^{cG}	05.10 ± 0.22^{bG}	980.45	≤0.001
	1	12.11 ± 0.64^{bF}	08.26 ± 0.54^{cF}	14.31 ± 0.28^{aF}	108.18	≤0.001
	2	27.79 ± 0.21^{bE}	20.09 ± 0.22^{cE}	30.18 ± 0.18^{aE}	2,001.89	≤0.001
	3	43.47 ± 0.28^{bD}	31.91 ± 0.14^{cD}	46.04 ± 0.10^{aD}	4,827.81	≤0.001
	4	62.33 ± 0.5^{bC}	52.07 ± 0.04^{cC}	66.14 ± 0.13^{aC}	1,310.04	≤0.001
	5	81.19 ± 1.03^{bB}	72.22 ± 0.06^{cB}	86.24 ± 0.18^{aB}	413.97	≤0.001
	6	100.05 ± 1.50^{bA}	92.37 ± 0.16^{cA}	106.33 ± 0.22^{aA}	191.23	≤0.001
F		5,230.01	5,8747.0	109,820		
P		≤0.001	≤0.001	≤0.001		

* Sample size (n = 3); standard deviation is given after ±; in line.

† For each parameter, mean values followed by the different superscript letters (a–c) were significantly different based on least significant difference (LSD) test at 0.05 level.

‡ For each maturity stage, mean values followed by the same superscript letters (A–G) were significantly different based on LSD test at 0.05 level.

Based on the essential fatty acid composition, nutritive value of LSO may be considered higher than that of peanut, palm and olive oils. Low PUFA/SFA ratio and Cox values of peanut, palm and olive oils observed indicate that the oxidative stability of these oils is higher than that of LSOs. Although LSO has a good nutritive value, its applications will be limited unlike the other three oils and it will require care for its self-stability because it will be more susceptible to oxidation.

Oxidative Stability of LSO

LSO at the three stages of maturity initially showed low deterioration state (PV < 5 meq O_2/kg). However, at CPW, it was less deteriorated than other stages based on PV and Cox values obtained at day 0.

Peroxide, K_{232}, K_{268}, *p*-anisidine, and TOTOX values are parameters used to assess the oxidation state of oils. The evaluation of these parameters in LSOs stored at 60C revealed that they increased during storage. Under accelerated tests at 60C, LSOs oxidized significantly and were not stable. The best oxidative stability of LSO was obtained at CPW. Oil at CPW could not be used after 3 days of storage at 60C, while the other two became unacceptable for consumption after 2 days. The PVs on day 3 of the oil at CPW were within the limits that the Codex Alimentarius Commission had adopted as satisfactory for unrefined olive oil, while those of seed oil from berries at 50-DAFS and at CPW + S60 exceeded that limit. According to the Codex Alimentarius Commission, the PV for unrefined olive oil may reach a maximum of 20 meq/kg (FAO/WHO 1993).

Secondary oxidation products such as 2-alkenals and 2,4-dienals generated by hydroperoxide decomposition are responsible for rancid flavor (Abdulkarim *et al*. 2007). The fact that the *p*-AVs remained constant for oils at CPW could mean that such products were not formed unlike at 50-DAFS and CPW + S60. *p*-Anisidine was also used in combination with PV to assess the extent of oxidative rancidity. Rancid flavor was less pronounced in the oil at CPW than oils at 50-DAFS and CPW + S60; it was most detectable in oils at CPW + S60. This trend was in agreement with the results for

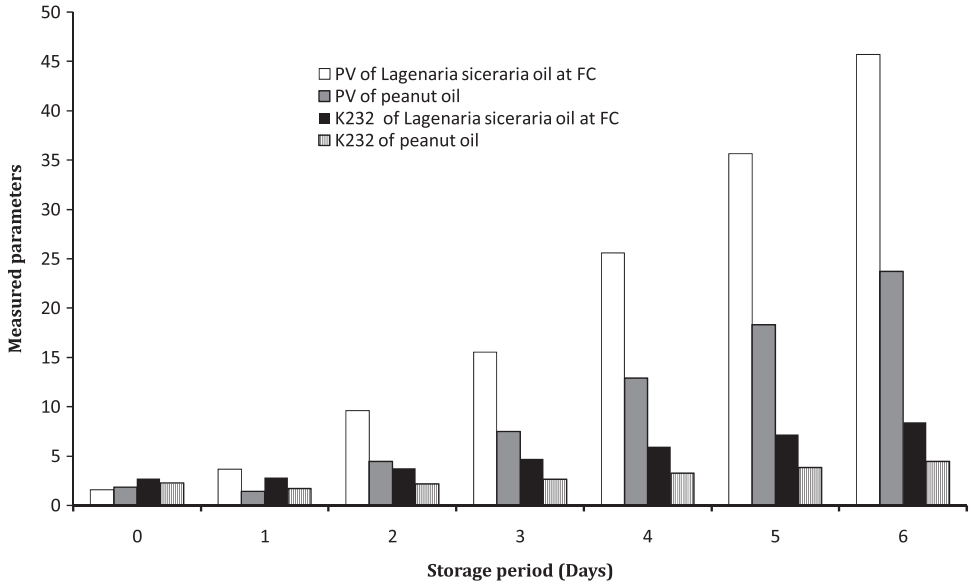

FIG. 1. CHANGES IN PV AND SPECIFIC EXTINCTION AT 232 (K_{232}) NM IN LSO AT CPW AND IN PEANUT OIL DURING 6 DAYS OF STORAGE AT 60C

PV, but it was different from that observed in the fatty acid composition. Oxidative stability of oil depends on other factors such as the amount and type of trace metals, natural antioxidants and the oilseed unsaturation (Smith *et al*. 2007). Specifications for food-grade oils usually indicated a maximum *p*-AV of 10 (Turner *et al*. 2006). Even if the three types of oil met this specification, the oil at CPW had developed fewer secondary oxidation products than the other two. *p*-AV of oil at CPW was lower than those of refined palm oil (2.3) indicated by the AOCS (1997).

When the unsaturated fatty acids were oxidized, conjugated fatty acids could be produced. These compounds could absorb UV light at 232 and at 268 nm. Conjugated dienes, the main products of linoleic hydroperoxide decomposition, and conjugated trienes, such as ketones, may be quantified by measuring specific extinction at 232 and 268 nm, respectively. K_{232} and K_{268} of *L. siceraria* at different stages were still low and correlated with the previously explained results. All the results confirmed that oil at CPW having low PUFAs had the highest oxidative stability. The results of this study were consistent with the previous studies on sunflower oils (Smith *et al*. 2007), showing that the oxidative and thermal stabilities of edible oils appeared to be related to linoleic and linolenic acid contents. Decreased linoleic and linolenic acid contents result in increased oil stability.

p-AV is often used in conjunction with PV to calculate the total oxidation or TOTOX. The lower TOTOX value of *L. siceraria* at CPW confirmed that the oil was more stable to oxidative rancidity and had a good resistance against heating than the other two.

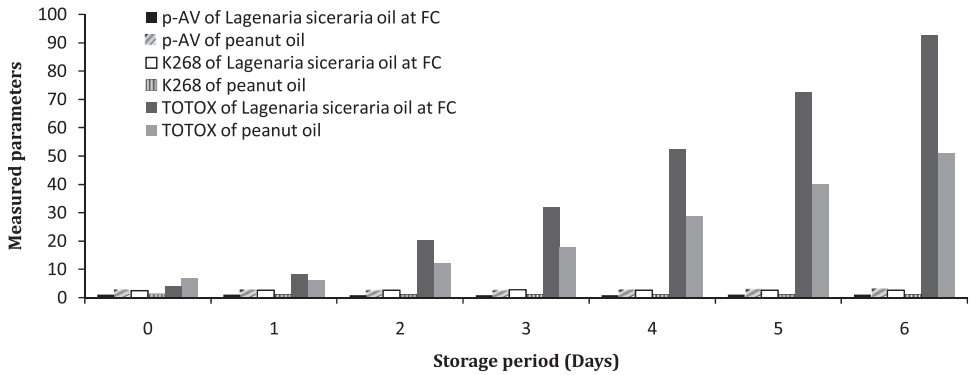

FIG. 2. CHANGES IN *p*-AV, SPECIFIC EXTINCTION AT 268 (K_{268}) NM AND TOTOX IN LSO AT CPW AND IN PEANUT OIL DURING 6 DAYS OF STORAGE AT 60C

Comparison of Oxidative Stability of *Lagenaria siceraria* and *Arachis hypogaea* Oils

According to the PVs, peanut oil was completely oxidized after 5 days of storage. This oil seemed more stable than LSO with the best oxidative stability (CPW). This could be explained by its high percentages of monounsaturated acids, as shown by the high level of oleic acids in peanut oil.

The formation of secondary oxidation products was expressed by an increase in *p*-AVs. Almost no change was observed from the beginning to the end of the experiment in *p*-AVs of the two types of oils. The secondary oxidation products (2-alkenals and 2,4-dienals compound) were low in LSO and peanut oil. Despite its low PVs, peanut oil had *p*-AVs (2.91–3.48) that were higher than those of LSO (0.87–0.93). *p*-AV in the initial oils (at day 0) may, however, be a property of both oils rather than the consequence of the process of oil oxidation. Indeed, some authors have indicated that *p*-AV was comparable only within each oil type because the initial *p*-AV varies among oil sources (Warner and Eskin 1995).

It is important to measure both the *p*-AVs and the PV when looking at oil quality as together they give a more accurate profile of the condition of the oil (Turner *et al*. 2006). Peanut oil that contained high percentages of monounsaturated acids exhibited lower levels of the conjugated dienes and trienes. The TOTOX value also confirmed the high oxidative stability of peanut oil compared with that of the LSO at CPW.

LSO was less stable than peanut oil, which, in turn, was less stable than palm and olive oils, respectively. However, the physicochemical properties of this oil (Loukou *et al*. 2011) meet the standards of Codex Alimentarius for edible oils. According to Matthäus *et al*. (2010), the roasting process had a positive effect on storage stability of the resulting oils. The stability of LSO could be improved by this process. *L. siceraria* crude oil, as the other crude vegetable oils, could contain undesirable components such as pigments, phosphatides, free fatty acids, off-flavors and off-odors. Therefore, the refining of the oil from roasted seeds of *L. siceraria*, by removing these contaminants, could increase its stability, improve its quality as well as maintain its nutritional values.

CONCLUSION

The present study indicated that oils extracted from seeds of *Lagenaria siceraria* berries at three different maturity stages were exposed to oxidation due to their high PUFA composition. Oil at CPW was more stable to oxidative rancidity and had better resistance against heating than oil at 50-DAFS and oil at CPW + S60. This oil could not be used after 3 days of storage at 60C. Monounsaturated peanut oil was more thermostable than LSO at CPW. The high degree of unsaturation shows a high nutritional value, but a high degree of instability to oxidation, which requires particular production and storage conditions.

NOMENCLATURE

LSO	*Lagenaria siceraria* oil
Cox	calculated oxidizability
PV	peroxide value
p-AV	*p*-anisidine value
K_{232}	specific extinction at 232 nm
K_{268}	specific extinction at 268 nm
TOTOX	total oxidation
50-DAFS	50 days after fruit set
CPW	at complete plant wilting
CPW+S60	after 60 days storage of fruit from plants wilted
PUFAs	polyunsaturated fatty acids
SFAs	saturated fatty acids
MUFAs	monounsaturated fatty acids

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