



Physico-chemical properties, fatty acid composition and storage stability of *Coula edulis* Bail. seed oil from Côte d'Ivoire

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Key words: Seed oil, *Coula edulis*, Physico-chemical properties, Fatty acids, storage.

<http://dx.doi.org/10.12692/ijb/8.5.190-201>

Article published on May 30, 2016

Abstract

Hazelnut (*Coula edulis* B.) is generally used as food in the south of Côte d'Ivoire. Some physico-chemical properties and fatty acid composition of *Coula edulis* seed oil were investigated in this study. Seed oil was soxhlet-extracted with hexan to yield a golden-yellow oil. *Coula edulis* seed is rich in oil (34.85%) and this oil showed a refractive index 1.46, acid value 2.65 mg KOH/g, free fatty acids as oleic acid 1.31%, peroxide value 9.10 meq O₂/kg, iodine value 91 g of I₂/100 g, saponification value 191.00 mg KOH/g and unsaponifiable matter 0.98%. The fatty acid composition of *Coula edulis* seed oil gave total saturated fatty acids 5.23% and unsaturated fatty acids 94.76%, and revealed that oleic acid (93.66%) and palmitic acid (3.29%) are the most abundant unsaturated and saturated fatty acids respectively. The physico-chemical changes occurring in *Coula edulis* seed oil during 90 days of storage at room temperature (26.56±3.00°C, 82.00±5.00% RH) were also followed. Excepted refractive index and iodine value, some physico-chemical properties such as acid value, peroxide value and saponification value varied significantly (P≤0.05) during storage time and remains lower than the norm. These results suggest that *Coula edulis* seed oil seems to be interesting for food and industrial purposes since this oil of good quality is stable during the 90 days of storage.

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Introduction

Coula edulis is one of several varieties of the African hazelnut belonging to the family Olacaceae that comprises 250 species (Mabberley, 1997). Only three genus (*Coula*, *Heisteria*, *Ximenia*) of this family are consumed by African populations (Aké-Assi, 1984). *Coula edulis* Bail. is a medium-sized, evergreen tree growing to a height of 25-38 m with dense crown (Bukola and Kola, 2008).

The fruit of *C. edulis* flowers between January and May (Davidson, 1999). It is described as a nut, ellipsoidal in shape, being about 3-4 cm long with flesh 5-6 mm thick surrounding the kernel (Davidson, 1999). The fleshy fruit is tasty but covered with a hard thick shell that makes the nut difficult to harvest and each drupe contains only one nut (Vivien and Faure, 1985). According to Bukola and Kola (2008), *C. edulis* is commonly known as African walnut or Gabon nut tree due to its edible seeds.

Coula edulis is a commonly occurring medicinal plant in Africa. The stem and fruits of this plant are commonly used in West Africa for the treatment of stomach ache and skin diseases. It has shown antibacterial and anti-yeasts activities (Bukola and Kola, 2008) and is also used as tonifiant (Iwu, 1993). The bark of *C. edulis* is used for dressing sores, to treat dysentery, to stimulate appetite (Duke, 2001) and to produce rinses or enemas for loin pains or kidney problems. It is also reported to contain acetylenes known to exhibit anticancer activity (Dembitsky, 2006).

Coula edulis enjoys wide acceptability as food because of its accessibility. Several ethnobotanical studies inventoried *C. edulis* as favorite food of Ivorian populations (Kouamé *et al.*, 2008). The nut has agreeable taste resembling hazelnut or chesnut (Bukola and Kola, 2008). Indeed, the hazelnut kernels consumed in different shapes constitute a good source of nutrients (Ekop and Eddy, 2005). Seeds are generally consumed after various processes like soaking, cooking, milling, roasting, puffing and germinating (Güzel and Sayar, 2012).

In Côte d'Ivoire, *C. edulis* is one of the seeds which is neglected, underutilized, underdeveloped and even going into extinct. However in Cameroon and Nigeria, it's used as food components (Tchiegang *et al.*, 1998). Nevertheless, it has been listed among the endangered plants producing edible fruits and seeds in southern part of Côte d'Ivoire. Fresh seeds of *C. edulis* contain 34.9% of fat and 38.6% of starch, while the raw flour contains 33.9% and 44.1% respectively and 604 mg/100 g of potassium and 393 mg/100 g of phosphorus (Ekissi *et al.*, 2005). Fatty acids containing a large proportion of oleic acid (95.5-97.4%) and triacylglycerides were found in the oil (Tchiegang *et al.*, 1998).

A lot of work has been carried out on analysis of seed oils, primarily because of extensive demands for oils both for human consumption and for industrial applications; consequently there is an increasing need to search for oils from non-conventional sources to augment the available ones and also to meet specific application (Kyari, 2008). Therefore, the need exists to look for other sources to supplement the supplies.

The aim of this study was to evaluate some physico-chemical characteristics and fatty acid composition of oil from hazelnut (*C. edulis*) seeds grown in Côte d'Ivoire. In addition, to determine the effect of storage conditions on oil stability. These will enable to exploit the nutritional and industrial potentialities of *C. edulis* oil seeds.

Materials and methods

Materials

Coula edulis fruits that have reached maturity were collected from a farm in Azaguié, South-East portion of Côte d'Ivoire (West Africa). Kernels were manually removed from seeds using a stainless steel knife, soaked in distilled water and washed thoroughly to free them of any adhering flesh. Then, the washed seeds were crushed into a paste using a previously cleaned laboratory mill and used to oil extraction.

All other chemicals, reagents and solvents used were of analytical grade and purchased from Sigma Chemical Co. (St. Louis, MO).

Methods

Oil extraction

Oil extraction from *C. edulis* seeds was carried out by four methods: Soxhlet extraction using petroleum ether or hexan (AOAC, 1990); Folch extraction using chloroform/methanol (2:1) (Folch *et al.*, 1957); and cooking method described by Gbogouri (2005).

Storage conditions

After removing solvent used in Soxhlet extraction method, using a Rotavapor apparatus, the extracted oil was weighted to determine the oil content of seeds. Oil samples were transferred to transparent glass bottles (100 mL of 5 cm in diameter). The bottles were closed and stored at room temperature in darkness under prevailing tropical ambient conditions ($26.56 \pm 3.00^\circ\text{C}$, $82.00 \pm 5.00\%$ RH). Samples of stored oil were collected at fixed time intervals (days 0, 15, 30, 45, 60, 75 and 90) for subsequent experiments.

Physical and chemical parameters of oil

The colour of the oil was noted using visual inspection at room temperature. Refractive index was determined according to AOAC Method (standard 921.08, 1997) at $25 \pm 0.05^\circ\text{C}$ with an automatic refractometer (RFM 81, multisecale).

Lipid index was analyzed using standard procedures: acid value and free fatty acids (standard 969.17; AOCS, 1997), iodine value (standard 993.20; AOCS, 1997), peroxide value (standard 965.33; AOCS, 1997), saponification value (standard 920.160; AOCS, 1997) and unsaponifiable matter (ISO 18609; Norme, 2000).

Fatty acid composition

The oil samples were transformed into fatty acid methyl esters (FAME) through transesterification according to Sukhija and Palmist (1988). FAME were analyzed using gas chromatography on HP-6890 system (Hewlett-Packard, CA, USA), equipped with a flame ionization detector (FID) and fused silica capillary HP-5 column (30 m x 0.32 mm x 0.25 μm ; cross-linked 5% PH ME Siloxane). Column temperatures were programmed from 200°C for 10 min, with a rise of $5^\circ\text{C}/\text{min}$ to 240°C for 10 min. The injector and detector were maintained at 240°C and 250°C , respectively. The sample volume injected on to the capillary column was 1 μL using splitless injection mode. Nitrogen gas at a flow rate of 1.2 mL/min was used as the mobile phase.

The unknown FAME were identified by comparing their retention times with those of pure standards and quantified on the basis of peak areas. The fatty acid composition was expressed as relative percentage of the total peak area.

Statistical analysis

All analyses reported in this study were carried out in triplicate. Statistical significance was established using Analysis of Variance (ANOVA) models. Mean comparison was carried out using Duncan's multiple range test ($P \leq 0.05$), with the help of the software STATISCA 7.1 (StatSoft Inc, Tulsa USA Headquarters).

Results and discussion

Oil extraction

The result of oil extraction from hazelnut (*C. edulis*) seeds using four different methods is shown in Table 1. Oil content of *C. edulis* seeds ranged between 28.31 to 34.85%.

Table 1. Hazelnut (*Coula edulis*) seed oil content through different extraction methods.

Method	Extraction yield (%)
Soxhlet (hexan)	34.85 ± 0.68
Soxhlet (petroleum ether)	34.10 ± 0.23
Folch <i>et al.</i> (1957)	34.75 ± 0.68
Cooking (Gbogouri <i>et al.</i> , 2005)	28.31 ± 0.81

Values are mean \pm standard deviation of triplicate determinations.

This result shows that seeds of *C. edulis* are richer in oil than most of the conventional oilseeds such as cotton (15-27%) (Orhevba and Efomah, 2012), soybean (14%) and palm fruit (20%) (Nzikou *et al.*, 2007), but has less oil compared to *Dacryodes edulis*

pulp (43.2%) (Dzondo-Gadet *et al.*, 2005). These variations between oil yields in seeds could be attributed to their cultivation climate, ripening stage, harvesting time and the extraction method employed (Egbekun and Ehieze, 1997).

Table 2. Physico-chemical properties of hazelnut (*Coula edulis*) seed oil.

Parameter	Content
Colour	Golden yellow
Refractive index (at 25°C)	1.46 ± 0.01
Acid value (mg KOH/g of oil)	2.65 ± 0.11
Free fatty acid (% of oleic acid)	1.31 ± 0.15
Peroxide value (meq O ₂ /kg of oil)	9.10 ± 0.02
Iodine value (g of I ₂ /100 g of oil)	91.00 ± 0.72
Saponification value (mg KOH/g of oil)	191.00 ± 0.72
Unsaponifiable matter (%)	0.98 ± 0.01

Values are mean ± standard deviation of triplicate determinations.

Hexan was also found to be the best solvent for oil extraction from *C. edulis*, since maximum oil extraction is obtained with Soxhlet method using hexan (34.85%). This justifies its significant use in oil extraction methods from most plant products (Tchiegang *et al.*, 1998). Therefore, only oil obtained by Soxhlet extraction with hexan was retained for the subsequent analyses in our work.

Physico-chemical properties

Table 2 shows some physico-chemical parameters of hazelnut (*C. edulis*) seed oil. The seed oil from *C. edulis*, soxhlet-extracted with hexan, had an attractive

golden yellow colour and is fluid at room temperature.

The refractive index is an indication of oil saturation level. It is not a useful property for specifying oils but it is useful purity criteria that can be used as a quality parameter in preliminary testing of the seed oils. It depends on their molecular weight, fatty acid chain length, degree of unsaturation, and degree of conjugation (Nichols and Sanderson, 2003). The refractive index of *C. edulis* seed oil at 25°C was found to be 1.46. This value is within the range of those reported for edible oils (Zoué *et al.*, 2012).

Table 3. Fatty acid composition of hazelnut (*Coula edulis*) seed oil.

Fatty acid	Number of carbon	Content (%)
Caprylic	C8:0	1.03 ± 0.02
Capric	C10:0	0.06 ± 0.01
Lauric	C12:0	0.08 ± 0.01
Myristic	C14:0	0.42 ± 0.02
Palmitic	C16:0	3.29 ± 0.03
Palmitoleic	C16:1	0.05 ± 0.01
Stearic	C18:0	0.35 ± 0.01
Oleic	C18:1	93.66 ± 1.03
Linoleic	C18:2	1.05 ± 0.01
Saturated fatty acids (SFA)		5.23
Monounsaturated fatty acids (MUFA)		93.71
Polyunsaturated fatty acids (PUFA)		1.05

Values are mean ± standard deviation of triplicate determinations.

The oil is less thick compared with most drying oils whose refractive indices were between 1.48 and 1.49 (N'guetta *et al.*, 2015).

The acid value of *C. edulis* seed oil was 2.65 mg KOH/g. Acid value and percentage free fatty acids (FFA) are used as indicator of the edibility of oil.

These two parameters determine the use of oil for either edible or industrial utility. Acid value of the oil suitable for edible purpose should not exceed 4 mg KOH/g (Codex, 1992; Peace and Aladesanmi, 2008). The low acid value obtained indicates that *C. edulis* seed oil can be considered as fit for use as edible oil (Peace and Aladesanmi, 2008).

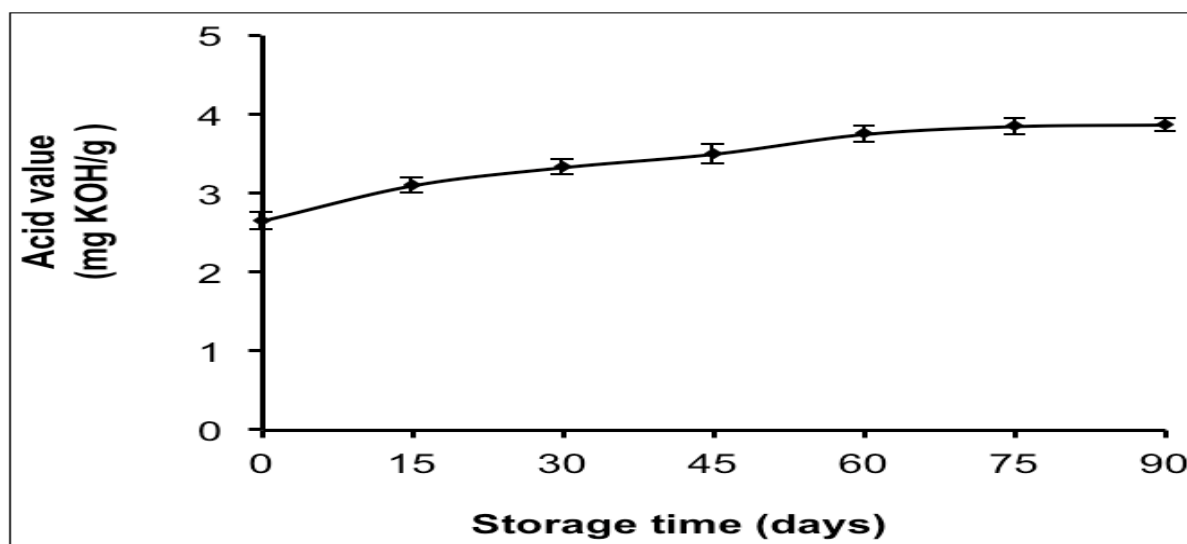


Fig. 1. Changes in acid value of *Coula edulis* seed oil during storage.

FFA content as oleic acid in *C. edulis* seed oil was found to be 1.31%. Oils usually contain small amounts of FFA such that when exposed to the air, these fatty acids, which are responsible for the acidity and oxidability of oils, produce unpleasant odours. The acid index measures the amount of these FFA in oil, which can be used as a measure of its quality. In refined vegetable oils, the lower the FFA content the more stable the oil, the more acceptable the oil to the human palate (Fokou *et al.*, 2009). The low percentage FFA content of *C. edulis* seed oil indicates that its can be stored for a long time without spoilage via oxidative rancidity (Ugbogu *et al.*, 2013).

Iodine value is a measure of the degree of unsaturation in oil and it is an identity characteristic of native oil. It indicates the degree of unsaturation in the fatty acids of triacylglycerol. This value could be used to quantify the amount of double bonds present in the oil, which reflects the susceptibility of oil to oxidation. The higher the iodine index, the more unsaturated the oil. The iodine value of *C. edulis* seed

oil is 91 g of I₂/100 g, suggesting that oil from *C. edulis* seeds is non-siccative (Hunter *et al.*, 2000). This value is higher than those of *D. edulis* pulp oil (60-85) (Kapseu and Parmentier, 1997), palm oil (50-55) and palm kernel oil (14-21) (Codex, 1993), but lower than those of unsaturated fatty acid-rich oils such as cottonseed (100-123), sunflower (118-141) and soybean (124-139%) (Codex, 1993).

However, this value was within the range of those obtained for *C. edulis* grown in Cameroon (90-95) (Tchiegang *et al.*, 1998) and those reported for edible oils (9.37-145) (Tan *et al.*, 2002). The high iodine value observed suggests the presence of high unsaturated fatty acids such as oleic and linoleic acid (Emil *et al.*, 2010). Moreover, this value is within the value of 120, which is an indication of its potential use as biodiesel feedstock (Knothe and Steidley, 2005).

The peroxide value measures the quantity of hydroperoxides in oil; it is used as an indicator of deterioration of oils. The lower the peroxide value, the

better the quality of the oil. Fresh oils have values less than 10 meq O₂/kg (Codex, 1992). The obtained peroxide value of *C. edulis* seed oil is 9.10 meq O₂/kg and indicates freshness of this oil. Similar result was found for sunflower oil (Tan *et al.*, 2002). According

to Ugbogu *et al.* (2013), peroxide value between 20 and 40 result to rancid taste. This suggests that oil from *C. edulis* seeds can be stored for long period without deterioration.

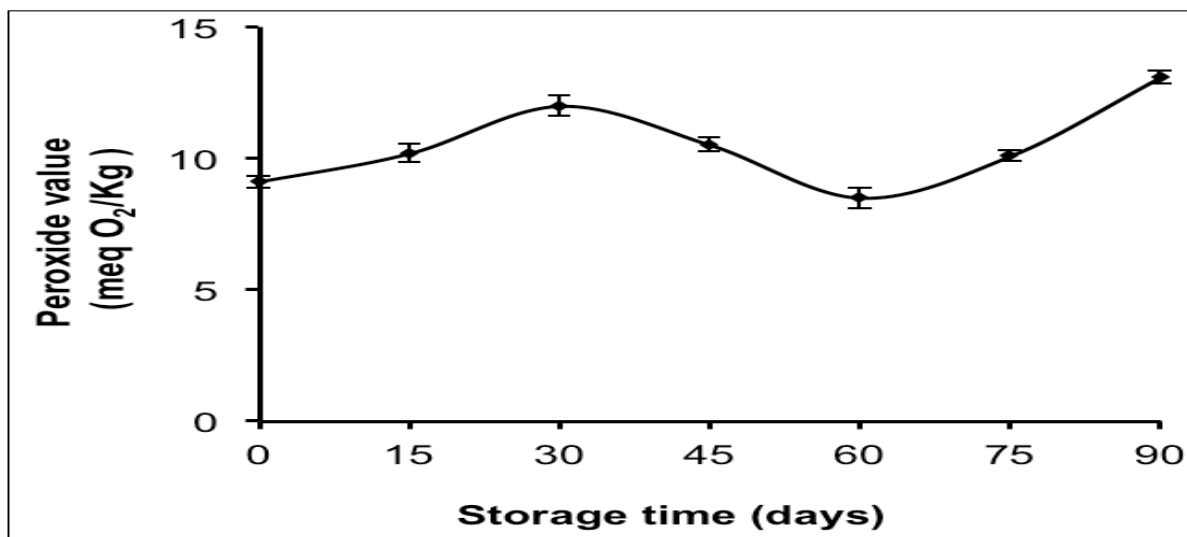


Fig. 2. Changes in peroxyde value of *Coula edulis* seed oil during storage.

The saponification value of *C. edulis* seed oil is 191 mg KOH/g and its unsaponifiable matter content is 0.98%. The saponification value compares favourably with usual oils such as cotton oil (189-198), soybean oil (189-195), peanut oil (187-196) and corn oil (187-195) (Codex, 1993). This value was also slightly higher than those of *C. edulis* grown in Cameroon (180-185) (Tchiegang *et al.*, 1998). The high saponification value obtained indicates that *C. edulis* oil possesses

normal triglycerides and may be useful in the production of liquid soap, shampoos and lather shaving creams (Ugbogu *et al.*, 2013). Also, the unsaponifiable matter of *C. edulis* seed is higher than those reported for other potential cosmetic oils such as cotton seed oil (0.52%), peanut oil (0.33%) and palm kernel oil (0.22%) (Kapseu and Parmentier, 1997).

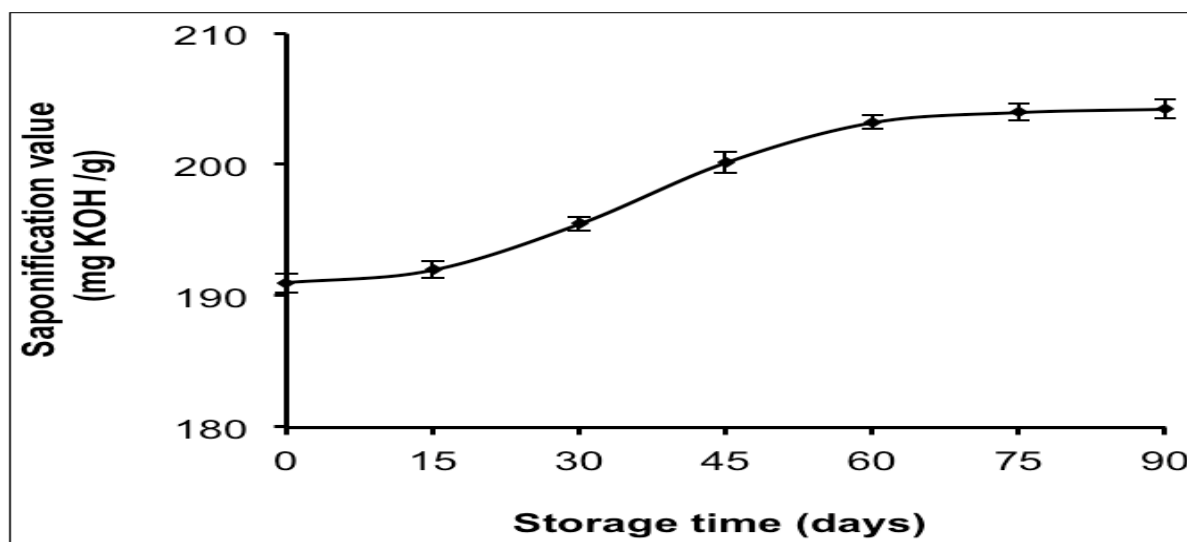


Fig. 3. Changes in saponification value of *Coula edulis* seed oil during storage.

Fatty acid composition

Fatty acid composition is a major determinant of oilseed crop quality. The fatty acid composition of *C. edulis* seed oil is summarized in Table 3 and shows nine identified fatty acids (C8 to C18). The result indicated that *C. edulis* seeds contained the high content of monounsaturated fatty acids (MUFA;

93.71%) and the low percentage of polyunsaturated fatty acid (PUFA; 1.05%) and saturated fatty acids (SFA; 5.23%). The MUFA content of *C. edulis* was higher than that reported for sunflower (23.2%), canola (61.5%) and olive (61.9%) (Bruzzetti, 1999). However, its PUFA concentration was lower than all mentioned species (Bruzzetti, 1999).

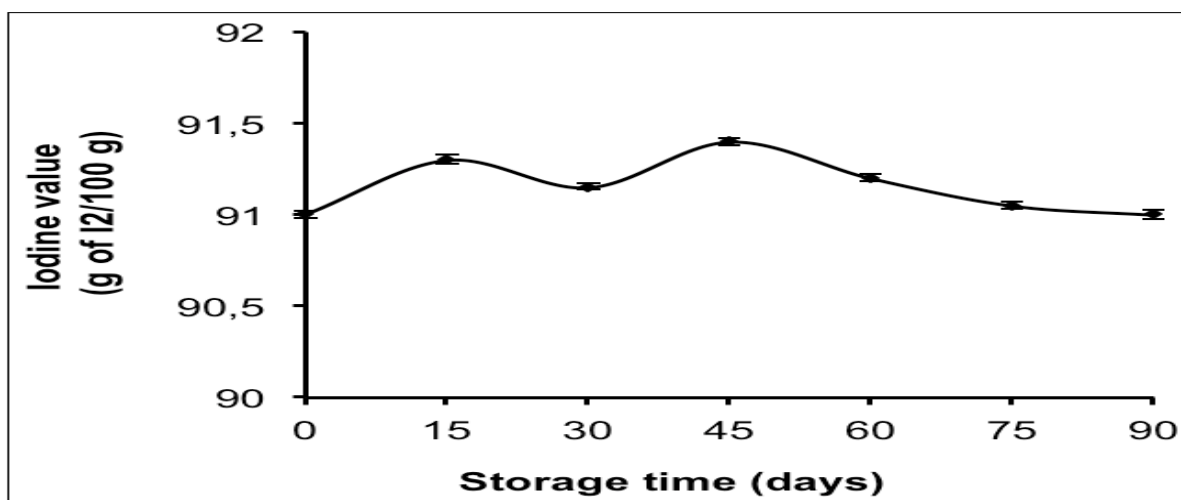


Fig. 4. Changes in iodine value of *Coula edulis* seed oil during storage.

Oleic acid (C18:1) with 93.66% was the predominant unsaturated fatty acid followed by linoleic acid (C18:2) being 1.05%. Palmitic acid (C16:0) was the major saturated fatty acid with 3.29% followed by caprylic acid (C8:0) being 1.03%. These results were comparable with those previously found by Moore and Knauff (1989), who reported that palmitic, oleic and linoleic acids constitute approximately 90% of the total fatty acid composition in peanut. Similar results were also found in *C. edulis* nuts from Cameroon (Tchiegang *et al.*, 1998).

The high proportion in oleic acid (ω -9) permits to affirm that *C. edulis* seed oil is essentially an oleic oil. The detected unsaturated fatty acids confer a dietary and an industrial importance to *C. edulis* seeds. Indeed, oleic acid has many beneficial effects on human health like decrease LDL levels in blood, suppress tumorigenesis, ameliorate inflammatory diseases and decrease blood pressure (Dhakal *et al.*, 2014). Many studies reported the least negative effect of oleic acid in relative carcinogenicity of fatty acids (Dallongéville *et al.*, 2008). It is known that the

excessive consumption of SFA is related to the increase of the plasmatic cholesterol and the obesity (Sales *et al.*, 2005). On the other hand, the consumption of PUFA and MUFA has been recommended to improve the lipidic profile in relation to the SFA. However, Eritsland (2000) indicates that rich diets in PUFA are not excepted of negative effects, having been demonstrated that those fatty acids may provoke an increase in the LDL ("bad") cholesterol oxidation and reduction of the HDL ("good") cholesterol levels. There is a tendency in increasing the recommendations of MUFA consumption, that seems to increase the HDL levels, and also it may act reducing the LDL and triacylglycerol blood levels, that makes it more effective in the prevention of heart diseases (Sales *et al.*, 2005). In lipochimie and specifically in industrial lubricants, MUFA are more appreciated because of their high fluidity, their weaker oxydability and stability (Jacotot, 1994).

Linoleic acid is an 'essential fatty acid' from which the whole ω -6 fatty acid family is derived. It is

indispensable for the healthy growth of human skin (Bruckert, 2001). Moreover, linoleic acid is important component of the cell membranes and is precursor of other substances involved in many physiological responses (Lai *et al.*, 2015). However, the low content in linoleic acid of *C. edulis* seed oil, would be an advantage because this fatty acid is rapidly oxidized and is considered the main cause of the abnormal flavors in oils.

Physico-chemical properties changes during storage

The effect of storage conditions on the physicochemical properties of *C. edulis* seed oil has been followed in order to study its stability. Fig 1 shows that acid value of *C. edulis* seed oil ranged from 2.65 to 3.87 mg KOH/g of oil and appeared to slightly increase significantly ($P \leq 0.05$) during the storage time.

This increase might be due to oxidation of unsaturated fatty acids under the influence of light and oxygen (Cheftel and Cheftel, 1984). However, after 90 days of storage in darkness at room temperature, the acid value obtained for *C. edulis* seed oil remains lower than the recommended value (4 mg KOH/g) of Codex Alimentarius (Codex, 1992) for edible oil. Furthermore, the oil quality is not considerably altered during storage time.

The peroxide value of the *C. edulis* seed oil stored in darkness at room temperature is presented in Fig 2. Storage time led to increase significantly ($P \leq 0.05$) the peroxide value from 9.10 to 12.00 meq O_2 /kg between 0 to 30 days of storage.

This increase might be attributed to the initiation of the oxidation in which there would be free radicals oxidation under the influence of prooxidant agents (metal traces and water), oxygen and light (Krishnamurthy, 1982). Fig 2 shows also that after 60 days of storage, the peroxide value of the *C. edulis* oil falls to reach 8.50 meq O_2 /kg, and that after 90 days of storage it reached 13.10 meq O_2 /kg. The reduction observed would result from the peroxides decomposition for the formation of free radicals or

by-products from oxidation (second initiation) (Tchiégang *et al.*, 2004). However, the second phase of increase observed after 60 days of storage would be due probably to the accumulation of the products derived from the peroxides decomposition. Indeed, the proliferation of these products enhances the oil oxidation without to need again an initiation phase (Tchiégang *et al.*, 2004). Despite this oil oxidation, the obtained peroxide value remains below the values (70 and 20 meq O_2 /kg) obtained for good oil (Wolff, 1991).

Fig 3 illustrates the evolution of the saponification value of *C. edulis* seed oil during storage time. The saponification value increases significantly ($P \leq 0.05$) during storage time and ranged from 191.00 to 204.25 mg KOH/g. This result could be due to a reduction in the length of fatty acid chains. Indeed, metal traces, light and oxygen could lead to cleave the unsaturated fatty acid chains during storage time which results in an increase in saponification value of *C. edulis* seed oil (Wolff, 1991).

It should be noted that during storage period studied, no significant ($P \leq 0.05$) changes in levels of iodine value and refractive index were observed (Fig 4 and 5). Similar results were found for *Riciodendron heudelotii* oils after 4 months storage at room temperature (Tchiégang *et al.*, 2004).

Conclusion

The results reported in the present work revealed that seeds of hazelnut (*C. edulis*) grown in Côte d'Ivoire have high oil content. Oil extracted from *C. edulis* seeds is unsaturated and has a nutritional value because it is rich in oleic acid.

The effect of storage conditions on the physico-chemical properties of *C. edulis* seed oil was also followed. No significant ($P \leq 0.05$) changes in levels of refractive index and iodine value were observed. However, acid value, peroxide value and saponification value varied significantly ($P \leq 0.05$) during storage time, but these values remained below the recommended limits. From these results, hazelnut

(*C. edulis*) seed oil exhibits a good quality and appears stable during 90 days of storage, making it a promising oil for nutritional and industrial applications.

Acknowledgement

This work was supported by Ph.D. grant to the first author. The authors are grateful to Laboratory of Biocatalysis and Bioprocessing at the University Nangui Abrogoua (Abidjan, Côte d'Ivoire) for technical assistance.

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