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Comparative study of seed roasting effects on *Sesamum radiatum* Schumach. & Thonn and *Sesamum indicum* L. oils quality

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ABSTRACT

Several species of sesame have been extensively studied but little is known about *Sesamum radiatum (SR)*. In this work, seeds roasting impact on two *Sesamum* spp, *SR* and *S. indicum (SI)* oils quality was studied. Physicochemical parameters (PP), fatty acids profile (FAL) and antioxidant activities were evaluated using spectrophotometry methods and gas chromatography coupled to mass spectrometry (GC-MS). Unroasted seeds (US) of *SR* oil extraction showed a yield of 40.26 ± 0.86% while roasted seeds (RS) at different temperatures (145-200°C) demonstrated a yield of 40.11 ± 1.36%. Evaluation of PP of oils according to the French Standardization Association (AFNOR) standards showed the following results: refractive index: 1.47435-1.47665 for *SR* and 1.46660-1.47065 for *SI*; acid number: 2.01-3.35 for RS against 10.04±0.53 mg KOH/g for US; saponification number: 186.82-191.53 for *SR* and 189.74-190.42 mg KOH/g for *SI*. Iodine value: 100.31-127.74 for *SR* against 117.96-128.04 g I_2/100 g for *SI*; peroxide value: 1.19-4.00 for *SR* against 1.66-2.58 meq/Kg active O_2 for *SI*. FAL was slightly modified during roasting showing that *SR* oil is an oleic-linoleic oil. More, *SR* oil demonstrated twice antioxidant activity than *SI*. These results demonstrated that roasting has positive effects on *SR* oil nutritional quality. *SR* oil could be recommended in food after toxicity studies.

Keywords: Sesame oils, Physicochemical parameters, Fatty acids, Antioxidant activity, Vegetable oil.

INTRODUCTION

Sesamum radiatum Schum. et Thonn. is a plant with high food, cosmetic and economic potential (Konan et al., 2012; Sene et al., 2018). As an annual oilseed crop well adapted to dry tropical areas, it has a bright future in Africa (Bedigian, 2003). Globally, sesame is generally found up to the 25th parallel on either side of the equator. It is also grown elsewhere, including China, the United States, Russia, Australia and South America. Its world production is estimated to 2 292 000 tons

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(Dale, 2001) with 1 628 000 and 538 000 tons from Asia and Africa, respectively. In general, exportations are made to Japan, Italy and Venezuela, where the product is perceived as an essential food source (Mkamilo et al., 2007).

Traditionally, oil is obtained from sesame seeds by pressing roasted seeds with an expeller (Martinez et al., 2017). Fatty acid composition of each *Sesame* spp. is specific, and defines its use. Sesame oil contains an average of 80% unsaturated fatty acids and many bioactive components, including tocopherols, phytosterols, and lignans such as sesamoline, sesamin, and sesamol (Lee et al., 2008; Komivi et al., 2018), thus resulting in relative stability against oxidative degradation. *In vivo*, some studies revealed that sesame oil contains lignans that reduce inflammation and oxidative stress (Alipoor et al., 2012). Sesaminol, one of the antioxidants in sesame oil, reduces lypopolysaccharide-induced oxidative stress and upregulates phosphatidylinositol 3-kinase/Akt/endothelial nitric oxide synthase pathways (Sankar et al., 2005; Alipoor et al., 2012). To avoid degradation of those components with medicinal properties, temperature pretreatment by roasting should be done carefully.

The purpose of roasting seeds before oil extraction is to change the microstructure, physical state and chemical composition. However, heat pretreatment should be controlled to prevent alteration of the oil quality (Gbogouri et al., 2011). It could damage proteins of sesame seeds and reduce nutritional value of sesame products. Furthermore, very high temperature could darken the sesame oil color and produce hazardous substances (Singh et al., 2016; Ji et al., 2019). It is also reported that polycyclic aromatic hydrocarbons levels increase in the oil at high temperature (Singh et al., 2016).

In West Africa, oil production has remained artisanal (Jensen et al., 2013). The use of seeds for food and production of derived products are still neglected because the oil consumption leads to digestive disorders if the seeds did not undergo rigorous thermal pretreatment. *S. radiatum* is currently classified among neglected and underutilized plants (Konan et al., 2012).

Because of its high nutritional and market values, sesame oil could contribute to food security for the local population going through many challenges including climate change. The present study was therefore aimed at: studying the impact of seeds roasting on the chemical composition, the quality and the physicochemical parameters of *S. radiatum* oil in comparison with the best known *Sesamum* specie *S. indicum*.

MATERIALS AND METHODS

Plant materials

Seeds of *Sesamum radiatum* and *Sesamum indicum* were collected in Bambouaka in the Northern part of Togo (West Africa) in October and November. The seeds were identified at the Biology Laboratory of the Faculty of Sciences of the University of Lome. Seeds were dried in laboratory at room temperature (25 ± 2°C). After drying, seeds were divided in five groups and roasted as presented in Table 1. After roasting, seeds were grounded to powder and introduced into a cotton cartridge for fat extraction.

Oil extraction

Soxhlet was used for solvent oil extraction. Sesame seed powders previously obtained were extracted with hexane for 8 hours per day for three days, following the standard protocol of NF V03-924. At the end of extraction, the solvent was evaporated under vacuum using a rotary evaporator system of Büchi type. Hexane solvent trace was eliminated in oven at 103±2°C. Oil samples were kept in stained glass vials and refrigerated (4°C) until used for experiments.

The oil extraction yield (R), was calculated by dividing the oil mass by the seeds mass.

Physicochemical parameters of sesame oils

Density, refractive index, acid value, acidity, saponification number, peroxide value and unsaponifiable matter content of oils were determined using the standard method described in NF T 60-214 (Afnor, 1984). Specifically, the refraction index measurement was carried out using an Abbé refractometer type "AZZOTA", equipped with a thermometer whose scale covers the measurement values from 20°C to 80°C. Naphthalene bromide was used as a standard to calibrate the reading. For the determination of the peroxide value, oils were diluted in acetic acid and chloroform and treated with a solution of potassium iodide. The released iodine was titrated with a sodium thiosulfate solution.

Additionally, water content and volatile matter were measured by drying a sample of oil at 103 ± 2°C in an oven at atmospheric pressure until a constant mass was obtained. Ester index was obtained by the difference between saponification index and acid number. Furthermore, the iodine value of oils (IV) was determined according to the method of Wijs (Novidzro et al., 2019). An excess of iodine chloride, called Wijs reagent, was added to the oil diluted in chloroform. After a few minutes of reaction, potassium iodide and distilled water were added. The produced iodine was titrated with a standard solution of sodium thiosulfate (0.1 N) in the presence of starch.

Preparation of fatty acid methyl esters (FAMEs)

Before gas chromatography coupled to mass spectrometry (GC-MS) analysis, oils fatty acid composition of *S. radiatum* and *S. indicum* oil were determined by derivatization to methyl esters (Ichihara et al., 2010). In a hemolysis tube with a screw cap, 20 mg of oil was solubilized in a 25% methanolic solution of sulfuric acid. The mixture was homogenized and then heated in an oven at 80°C for 90 min. Subsequently, a volume of 1.5 mL of sodium chloride solution (0.9%) was added to the mixture. After vigorous stirring, the final solution containing the fatty acid methyl esters was extracted with 0.5 mL of hexane. The fatty acid methyl esters were analyzed by GC-MS (Gas chromatography–mass spectrometry).

GC-MS quantification method

FAMEs were analyzed by a TRACE 1300 Series GC chromatograph with a split/splitless injector. Separations were achieved using a DB5-MS capillary column (50 m × 0.25 mm ID, 0.25 µm film thickness). Helium was used as the carrier gas at flow rates of 1 mL/min. The injector was a type of Auto sampler AIS/AS 1310 and kept at 250°C in a splitless mode The GC oven temperature was initially kept at 120°C for 10 min; then increased from 120 to 210°C, at a rate of 3°C/ min and kept constant at 210°C for 10 min, before increased to 300°C at a rate of 5°C/ min and kept isothermal for 2 min. A mass spectrograph (MS) ISQ MS was coupled to the GC with an ionization energy of 70 eV. Data was acquired with XCalibur software. MS spectra were obtained at range width m/z 40-450, interface temperature was 255°C, ion source temperature was 210°C, solvent delay was 3 min, and scan speed was 2500. FAME peaks were identified by comparing their retention time and equivalent chain length with respect to standard FAME. All determinations were carried out in triplicates

Oils antioxidant activity

2,2-Diphenyl-1-PicryHydrazine (DPPH) assay

The DPPH test was carried out as described before (Sid et al., 2018). One hundred and fifty microliters of various dilutions of each type of extract suitably diluted was added to 3 mL of a 10^{-4} M DPPH solution in methanol. After an incubation period of 30 min, the absorbance of the samples was read at 517 nm using a J Genesys 10S UV-Vis

Spectrophotometer (USA). Quercetin was used as positive control. Results determined from calibration curve equations were expressed as microgram quercetin equivalents per milligram oil (µg QE/mg).

Ferric Reducing Antioxidant Power (*FRAP*) *assay*

The procedure described by Nair et al., (2007) was followed. The principle of this method is based on the reduction of a ferric-tripyridyltriazine complex to its ferrous, colored form in the presence of antioxidants. Briefly, the FRAP reagent contained 2.5 mL of a 10 mmol/L TPTZ (2,4,6- tripyridy-s-triazine) solution in 40 mmol/L HCl plus 2.5 mL of 20 mmol/L $FeCl_3$ and 25 mL of 0.3 mol/L acetate buffer, pH 3.6 and was prepared freshly and warmed at 37°C. Aliquots of 100 µL sample supernatant were mixed with 3 mL of FRAP reagent and the absorbance of reaction mixture at 593 nm was measured spectrophotometrically (Genesys 10S UV-Vis Spectrophotometer, USA) after incubation at 37°C for 10 min. 1 mmol/L $FeSO_4$ was used as the standard solution. The result was expressed as µmol Eq $FeSO_4$/mg oil.

Mineral elements determination

Mineral elements (Mg, Ca, Na, K, Mn, Zn, Cd, Cu, Pb, Cr, Ni, As and Hg) were determined by flame atomic absorption spectrophotometry after mineralization. Oils mineralization was made by the wet process (acid etching). An atomic absorption spectrophotometer (brand iCE 3000 SERIES THERMO FISCHER), equipped with a Coupled Charge Device detector and a hydride generator VP100 were used for the analysis. Contents determination of mineral elements in oils was carried out with a pre-established calibration curve.

Statistical analyses

Statistical analysis was run using OriginPro 8.0 software with $P<0.05$; ESM n=3. Data were reported as mean value ± standard deviation of triplicate or duplicate analyses. When difference between duplicate analyses was greater than 10%, analysis was repeated. Data were subjected to analysis of variance (ANOVA).

Table 1: Sesamum seeds pretreatment at different temperatures.

Sample	**Treatment**
S. radiatum seeds S_1	Not roasted
S. radiatum seeds S_2	Roasted at 145 °C
S. radiatum seeds S_3	Roasted at 175 °C
S. radiatum seeds S_4	Roasted at 200 °C
S. indicum seeds S_5	Not roasted

RESULTS

Seeds oil yield

S. radiatum roasted seeds at 175°C demonstrated a yield of 40.11 ± 1.36 while the yield was 40.26 ± 0.86 for the unroasted seeds.

Organoleptic character of oils

The extracted oils were light yellow for unroasted seeds and dark yellow to brown-black for roasted seeds. All extracted oils were liquid at room temperature (25-32°C).

Oils physicochemical characteristics

Water and volatile matter content

For roasted seeds, 5.05% was found while 5.79% was obtained for unroasted seeds oils.

Acid number and acidity

Analysis of these two parameters on *S. radiatum* oils from seeds roasted at different temperatures (145 -200°C) and different times (25 and 45 min) are shown on Figure 1-a) and Figure 1-b).

Oils refractive index, density, saponification index, ester index, unsaponifiable content, peroxide and ester value

The results of these parameters are presented in **Table 2**.

Iodine number and refractive index

The iodine numbers are showed in Table 2 for the produced oils herein. Those numbers allow to classify the oils as semi-drying oils that iodine numbers are between 100 and 130 g I_2/100 g oil. As a control, *S. indicum* seeds oil was analyzed. Results reported in Table 2 are in the same range as the oils of *S. radiatum*.

Fatty acid profile of *S. radiatum* and *S. indicum*:

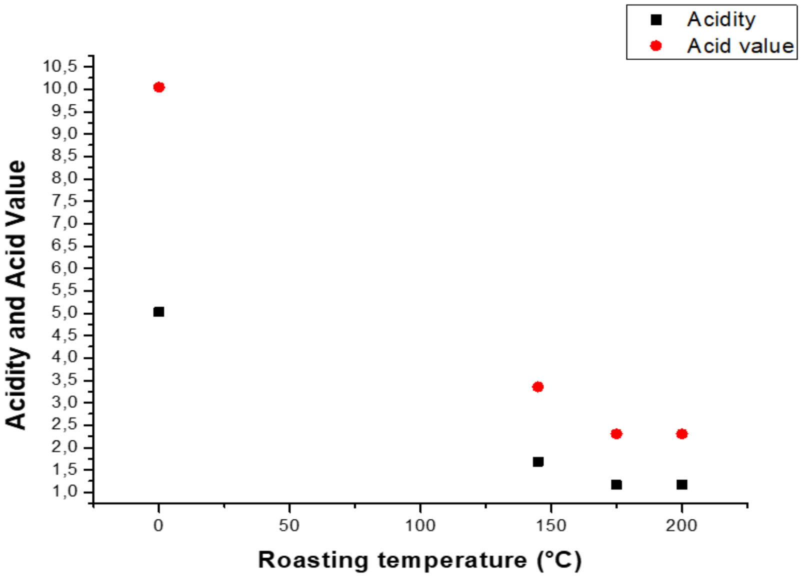
Fatty acids profile of oils produced and analyzed by GC-MS in this work is shown in Table 3. Palmitic (C16:0), palmitoleic (C16:1, ω_7), stearic (C18:0), oleic (C18:1, ω_9), linoleic (C18:2, ω_6) and arachidic (C20:0) fatty acids were the main fatty acids identified. Alpha-linolenic (C18:3, ω_3), eicosanic and erucic fatty acid were under the detection limit for both studied plant seeds oils.

Oils antioxidant activity

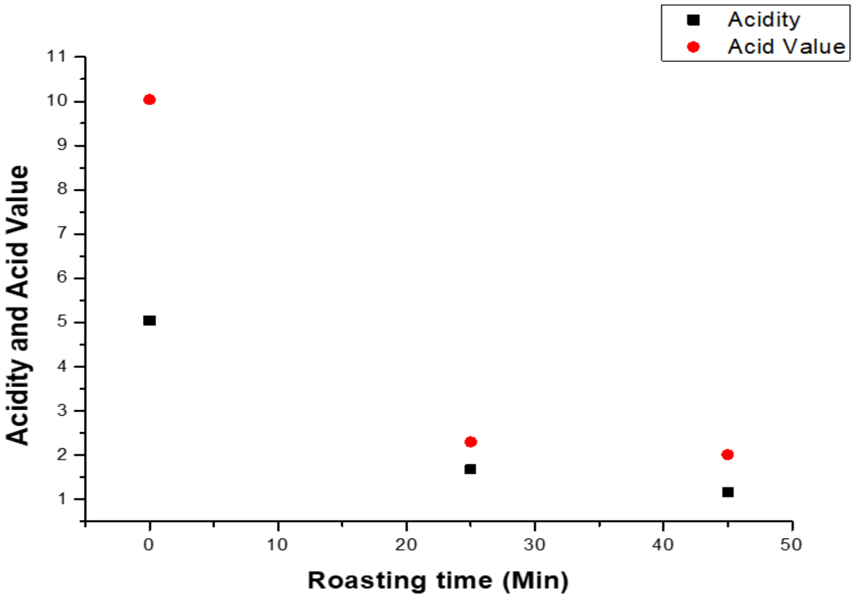
Results of antioxidant activity are shown in Figure 2. FRAP assay showed more antioxidant activity for roasted seeds oils than unroasted oils.

Metallic trace elements:

Results of metallic trace elements analysis are reported in Table 4. Calcium was the most present in *S. radiatum* oil followed by sodium. It was also found that Mg, Ca and Na levels decreased when seeds were roasted.



a)



b)

Figure 1: Evolution of acid number and acidity as a function of temperature (**a**) and time (**b**).

Table 2: Effects of roasting on refractive, peroxide, iodine and saponification indices.

Measured parameters	***S. radiatum* oil**		***S. indicum* oil**		**Codex Alimentarius**
	US	**RS**	**US**	**RS**	
Color and physical condition (room temperature)	Light yellow and Liquid	Dark yellow and liquid	Light yellow and Liquid	Dark yellow and liquid	-
Refraction value (nD, 20°C)	1.47435±0.00215	1.47520-1.47665	1.4666±0.0005	1.47065±0.00405	1.465-1.469 for *S. indicum* (*SI*)
Saponification number (mg KOH/g d'huile)	186.82±1.11	187.28-191.53	190.42±3.41	189.74±4.43	186-195 (*SI*)
Ester index (mg KOH/g d'huile)	176.78±0.40	184-189	181.27±2.02	185.93±3.05	-
Contents of unsaponifiables (%)	1.65±0.17	1.50±0.05	1.30±0.05	1.20±0.15	≤2% (*SI*)
Iodine value (g I_2 /100 g d'huile)	125.48±2.37	100.31-127.74	128.04±3.40	117.96±1.82	104-120 (*SI*)
Peroxide number (méq/ Kg d'O_2 active)	3.19±0.81	1.19-4.00	2.58±0.08	1.66±0.02	≤5 (virgin oil)

Table 3: Fatty acid methyl esters (FAMEs) chemical composition by chromatographic area percentages of unroasted seeds of *S. radiatum* and *S. indicum.*

Denomination	Fatty acids	Area percentage (%)	
		S. indicum	*S. radiatum*
Myristic acid	C14 :0	<0.1	0.21
Palmitic acid	C16 :0	6.44	13.85
Palmitoleic acid	C16 :1 ω^7	<0.1	0.21
Margaric acid	C17 :0	<0.1	ND*
Stearic acid	C18 :0	4.18	4.28
Oleic acid	C18 :1 ω^9	45.49	48.54
Linoleic acid	C18 :2 ω^6	41.94	32.66
Linolenic acid	C18 :3 ω^3	ND	ND
Arachidic acid	C20 :0	<0.10	0.10
Gondoic acid	C20 :1	<0.10	ND
Behenic acid	C22 :0	<0.10	ND
Saturated Fatty Acids (SFAs)		**10.62**	**18.44**
Monounsaturated Fatty Acids (MUFAs)		45.49	48.75
Polyunsaturated Fatty Acids (PUFAs)		41.94	32.76
Unsaturated Fatty Acids (UFAs)		**87.43**	**81.51**
(MUFA+PUFA) /SFA		**8.23**	**4.42**

*ND : Not determined

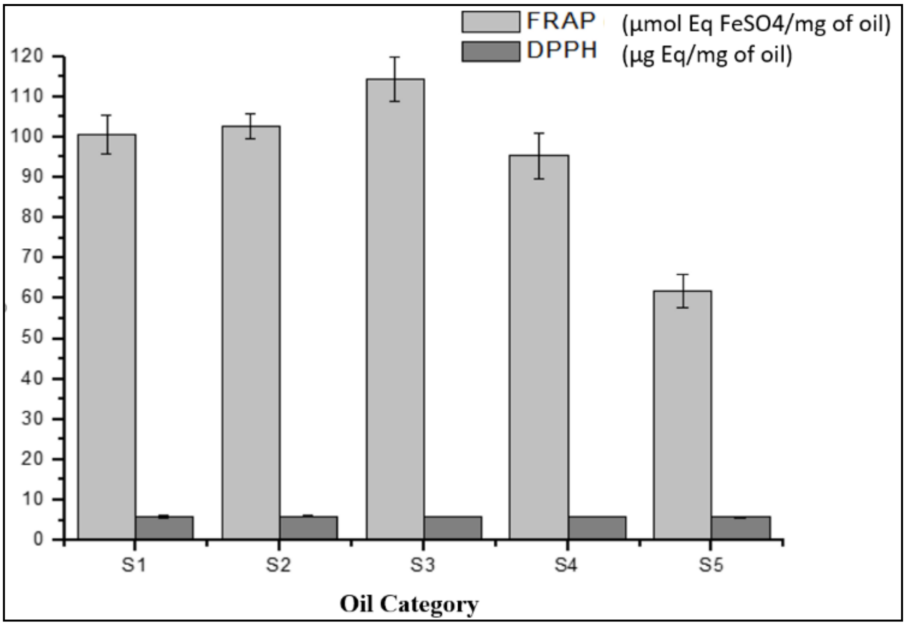


Figure 2: Antioxidant capacities in µg EQ/mg oil (for the DPPH radical inhibition method) and µmol Eq $FeSO_4$/mg oil (for the FRAP method). The values are expressed as an average ± ESM; n= 3.

Table 4: Trace elements (metals) in the *S. radiatum* oils investigated.

Metal Traces elements	***S. radiatum* (Concentration en mg/L)**	
	RS	**US**
Sodium (Na)	37.40±0.38	40.80±0.16
Magnésium (Mg)	26.90±0.17	38±0.23
Potassium (K)	2.35±0.01	2.02±0.03
Calcium (Ca)	71.40±0.05	99.96±0.21
Chromium (Cr)	0.08±0.01	0.11±0.00
Manganese (Mn)	0.31±0.05	2.63±0.02
Nickel (Ni)	0.23±0.01	0.28±0.01
Copper (Cu)	0.13±0.01	0.20±0.00
Zinc (Zn)	0.53±0.00	0.76±0.01
Cadmium (Cd)	< 0.0005	< 0.0005
Arsenic (As)	< 0.05 (µg/L)	< 0.05 (µg/L)
Mercury (Hg)	< 0.05 (µg/L)	< 0.05 (µg/L)
Lead (Pb)	< 0.01	< 0.01

The values are expressed as an average ± ESM; n= 2. All bold values on the same line are significantly different at $p \leq 0.05$.

DISCUSSION

Seeds Oil yield

Few works reported the yield of *S. radiatum*. When Pathak et al. (2020) extracted oil from the seeds, a yield of 29% was found. If the most known Sesamum specie *S. indicum* usually shows a yield over 50% (Akladious et al., 2018), with the increasing demands of fats and oils, *S. radiatum* oil yield is enough to be valorized. Improving oil extraction method could also result in yield increasing.

Organoleptic character of oils

The evolution of color is generally used to measure the degree of roasting (Manzocco et al., 2000). Having extracted oils from seeds roasted at different temperatures (0-200°C), different colors of oils were obtained. Similar results were found by Kandji (2001) on the organoleptic characteristics of sesame oil. Formation of brown substances after roasting results from non-enzymatic Maillard-type reactions between reducing sugars and free amino acids or amides (Guia, 2013). Our preliminary experiments also demonstrated that temperatures above 220°C resulted in sesame seeds oils with a black, charred appearance and a bitter taste.

Oils physicochemical characteristics

Water and volatile matter content

High moisture content in vegetable fats and oils generally leads to increased microbial load as well as lipid oxidation and rancidity. In this work, roasted seeds at 175°C and unroasted seeds were used for water and volatile matter contents determination in oils. The values found herein were slightly higher than the standards of Codex Alimentarius (Alimentarius, 2019) for virgin and unrefined vegetable oils, that is lower than 5%. To avoid early rancidity of *S. radiatum* oils extracted, an additional dehydration process is needed before storage

Acid number and acidity

Acid number of fatty substances is a good indicator to determine its alteration. It could be deduced from our results that roasting increase acid number and acidity that are linked to oils quality. Furthermore, those values are within the range of Codex Alimentarius standards (Alimentarius 2017) (≤5 mg KOH/g). Unroasted seeds oils, on the other hand, showed lower acidity and must require refining to be used as food and avoid subsequent denaturation.

At the best of our knowledge, this work is the first report of acidity measurement of *S. radiatum* oil. Hydrolysis and oxidation are consequences of poor oil conservation. Acid number measures the amount of free fatty acids resulting from hydrolytic reactions of triglycerides, making it a quality criterion to report conservation status of an oil (Kpegba et al., 2017). Kandji et al. (2001) found an acid number of 2.60 for *S. indicum* L oil that are close to our values for *S. radiatum*. A good quality oil according to the standards (Alimentarius, 2019), should have a near to zero acidity. Results demonstrated the importance to roast seeds until the obtention of a near zero acidity and simultaneously paying attention not to modify the length of free fatty acids (FFA) chains. The roasting process favors the enzymatic esterification reaction of the FFAs in the oil that participated in the decrease of the free acidity oleic acid.

Oils refractive index, density, saponification index, ester index, unsaponifiable content, peroxide and ester value

Iodine number and refractive index

Iodine number is directly related to the degree of unsaturation or number of double bonds in an oil. The more unsaturated an oil is, the higher its iodine value (Novidzro, 2019). Iodine number is used to assess the rancidity of oil, since the more unsaturations it contains, the more sensitive it is to oxygen. Based on the relatively high iodine value of *S. radiatum* oils measured, it could be suggested that those oils should be stored in such a way to avoid auto-oxidation. However, *S. radiatum* oils are rich at sesaminol, a strong antioxidant that could prevent the oil from oxidation (Lee et al., 2010).

Seeds oils consist of many different compounds. Their varying composition allows for them to be characterized by refractive index measurement. These measurements can therefore also provide insight into the quality of oils, as any change in their optimal composition will affect the refractive index. Refractive index is considered a criterion of purity of an oil. This index is proportional to the molecular weight of fatty acids and their degree of unsaturation. Refractive index values of *S. radiatum* oils produced in this work are shown in Table 2. Values found here (1.46-1.47) are similar to most of reported value for sesame oil (Novidzro et al., 2019) and could be classified as a semi-drying oil. Results found for *S. indicum* used as control were in the same range as the oils of *S. radiatum*.

Fatty acids profile of *S. radiatum* and *S. indicum*

Oils in the diet are available to the body as fatty acids, which are excellent sources of dietary calorie intake. A high intake of saturated fatty acids and cholesterol in the diet may lead to hypercholesterolaemia and a large intake of polyunsaturated fatty acids have a hypocholesterolaemic effect in human. Ji et al. (2019) reported similar fatty acids composition (Table 3) as our results. *S. radiatum* and *S. indicum* showed over 80% of unsaturated fatty acids with more saturated fatty acids in *S. radiatum*. When *S. radiatum* oil showed 18% of saturated fatty acids in this work, Orsavova et al. (2015) reported a percentage of 22% for *S. indicum* oil. Values reported herein could be used to calculated the maximal values of recommended daily energy intakes for polyunsaturated fatty acids (PUFAs) by using *S. radiatum* oil. A previous study on *S. radiatum* harvested in Tunisia showed seeds oil content of 44.85% monounsaturated fatty acids (MUFAs) and 39.68% of PUFAs (Ksouda et al

2018), demonstrating a similar result to our finding.

Oils antioxidant activity

Oil consumption could provide antioxidant intake at different level. Antioxidant compounds reduce free radicals and prevent damages to cellular membrane, mitochondria, and DNA, with beneficial effects on aging and cancer risk. We investigated antioxidant activity of oils produced using FRAP and DPPH assays. Apart from fatty acids, sesame oil contains fat-soluble vitamins and antioxidants. Among those compounds, vitamin E, vitamin A, tocotrienol and phytosterols were reported (Gafour et al., 2020). From the Figure 2, it clearly appears that *S. radiatum* has a greater antioxidant effect than *S. indicum* by 2 folds. Finally, the results revealed that roasting promotes the effect of antioxidant activity and the seeds roasted at 175°C and 200°C at 25 min showed the best antioxidant activity. Similar results interpreted by the conversion of sesaminol to sesamol and sesamolin to sesaminol and other lignans in sesame oil during seed roasting were reported by Lee et al. (2008).

Metallic trace elements

The quality of edible oils regarding their freshness, storability and toxicity can be evaluated by the determination of several trace metals. Some of them, such as Cu and Mn can increase the rate of oil oxidation. On the other hand, elements such as Cd, Cr, Hg, Ag and Pb are very important on account of their toxicity and metabolic role (Llorent-Martínez et al., 2011). Produced oils herein were analyzed for their metal trace elements composition using atomic absorption spectrophotometry. It was found that Mg, Ca and Na levels decreased when seeds are roasted. This drop could be explained by the reduced acidity due to roasting hence, giving a better-quality oil (Silva et al., 2013). Mostly, the amounts of trace elements found in oils are below hazardous level for consumption.

Conclusion

In this paper, the impact of seeds roasting on quality, fatty acid profile and physicochemical parameters of *S. radiatum* oil was studied. Results showed that *S. radiatum* oil is a good edible oil when seeds are roasted. More, water and volatile matter content, acid value, acidity and fatty acid profile showed that pre-refining and conditioning precautions must be taken for the unroasted seeds oil to limit degradation of oil quality. It was found out that roasting did not significantly modify the quantity of free fatty acids. From our findings, we could suggest *S. radiatum* oil be used in food, cosmetics and pharmacology after additional studies to evaluate amino acids, lignans, vitamins and other minerals contents and toxicology.

COMPETING INTERESTS

The authors declare that there is no competing interests in relation to this article.

AUTHORS' CONTRIBUTIONS

EBP, PB and KK designed the project. EBP, KE, and KD performed the sampling. EBP, KD, KBA, and LK performed the experiments; EBP, KD, and KE analyzed the data. EBP, KE, KSE, and KIS wrote the manuscript. KSE, KIS, FMA and KE supervised all activities. All authors agreed to the final version of the manuscript and to its publication.

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