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Research article

Study on antioxidant activity of crude peanut oils and refined cottonseed oils from Burkina Faso

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Abstract: Vegetable oils are among the foodstuffs produced and consumed in abundance by the population in Burkina Faso. These edible oils are nutrient sources for health. However, the oxidation of edible oils is a phenomenon that leads to their degradation. Hence, the objectives of this study were to determine the antioxidants compounds, to evaluate the antioxidant activity of vegetable oils produced in Burkina Faso and to propose good manufacturing practices. Thus, 32 samples of crude peanut oils and refined cottonseed oils were analyzed. α-Tocopherol was determined by High performance liquid chromatography (HPLC), total phenolic compounds and DPPH by spectrophotometry. The α-tocopherol averages are 10.89 and 56.44 mg/100 g for peanut oils and cottonseed oils, respectively ($p < 0.05$). For total phenolic compounds, the averages are 2.91 and 0.64 mg/100 g of gallic acid equivalent for peanut oils and cottonseed oils, respectively ($p < 0.05$). The inhibition percentages are respectively 17.97% and 5.58% ($p < 0.05$) for peanut oils and cottonseed oils. For antioxidant activity, the averages are 0.81 and 0.27 mg trolox/100 g for peanut oils and cottonseed oils, respectively ($p < 0.05$). Cottonseed oils have the highest levels of α-tocopherol while peanut oils have high levels of total phenolic compounds and antioxidant activity. Vegetable oils contain acceptable levels of α-tocopherol and total phenolic compounds for their oxidative stability and health benefits for the consumer. However, the levels of biomolecules will be higher if production and preservation conditions are improved and controlled. The results could be used for future recommendations about human feeding programs in Burkina Faso.

Keywords: vegetable oils; antioxidants; total phenolic; α-tocopherol; Burkina Faso

Abbreviations: HPLC: high performance liquid chromatography; DAD: diode array detector; PTFE: polytetrafluoroethylenen; DPPH: 2, 2-diphenyl-1-picrylhydrazyl; SPSS: statistical package for the social sciences; OP: Ouagadougou-Pabré; OS: Ouagadougou-Saaba; BD: Bobo Dioulasso

1. Introduction

Edible oils are foodstuffs that are derived from several raw materials. These oils are used in human food. They are beneficial and have an essential role in food in terms of technology, nutrition and organoleptic activity [1]. However, free radicals are highly toxic molecules found in most foods [2]. Indeed, the organism faces the attacks of these free radicals. These compounds are responsible for cellular and tissue damage that is often irreversible. The most vulnerable biological targets are proteins, lipids and deoxyribonucleic acid [2]. They are considered to be promoters of several diseases, including cancer, cardiovascular and neurological disease [3]. For this reason, the consumption of foods with a high free radical scavenging activity such as wine, fruits and virgin oils is strongly recommended [4]. The edible oils are one of the main sources of α-tocopherol [5] and phenolic compounds. In addition to their beneficial role for the body, these biomolecules contribute to the oil stability against oxidation [6].

Vitamin E is a general term used to refer to tocopherols and tocotrienols [7]. Tocopherols are the main group of primary antioxidants found in vegetable oils and fats [8]. The most common and biologically active tocopherol in nature is α-tocopherol. These compounds function as the most effective fat-soluble antioxidants, protecting cell membranes from pyroxylated radicals and mutagenic nitrogen oxide species [9]. Increased α-tocopherol intake was inversely associated with decreased risk of cardiovascular and coronary heart disease [10]. Other group of antioxidants known are Phenolic compounds. Phenolic compounds are different families and found in oils [11]. They are improperly called "polyphenols" and are responsible for the good stability against the oil oxidation. Also, these constituents are called bio-phenols [6]. The undeniable bio-phenols role are powerful antioxidants in the prevention of human pathologies [12,13]. The presence of antioxidant compounds makes it possible to determine the antioxidant activity of vegetable oil.

In Burkina Faso, refined cottonseed oils and crude peanut oils are produced for human consumption. These oils face competition from imported oils. However, no major studies have evaluated the biomolecules and antioxidant activity of these oils. Malnutrition and micronutrient deficiencies are a major public health problem in most West African countries [14] including Burkina Faso. Thus, knowledge of the levels of α-tocopherol, total phenolic compounds and oxidative stability would be an undeniable asset in terms of nutrition, production and consumption of edible oils. Hence, the objective of this study were to determine the antioxidants compounds, to evaluate the antioxidant activity of vegetable oils produced in Burkina Faso and to propose good manufacturing practices.

2. Materials and methods

2.1. Reagents

The reagents and solvents used for the determination of α-tocopherol, total phenolic compounds and antioxidant activity were HPLC grade. The standards of α-tocopherol (Sigma Aldrich, purity ≥ 96%), gallic acid (Sigma Aldrich, purity ≥ 99%, Cas 5995-86-8), trolox (Sigma Aldrich, Cas 53188-07-1, purty 97%), DPPH (Sigma Aldrich, Cas 1898-66-4) and ammonium acetate (purity 98%), Sodium carbonate (Sigma Aldrich, purity ≥ 99,5%, Cas 497-19-8) were used. Methanol (Fisher scientific, purity ≥ 99%), tetrahydrofuran (Fisher scientific, 99% extra pure) are HPLC grade, Folin-Ciocalteu reagent (AppliChem, Germany) were used for total phenolic compounds determination.

2.2. Samples collection

The oil samples collected were crude peanut oils and refined cottonseed oils. A total of thirty-two (32) samples were collected in 60 mL amber plastic vials and hermetically sealed and stored in icebox for future analysis. Crude peanut oils were collected from production sites and some markets. Refined cottonseed oils were collected from production units in Ouagadougou, Pabré and Bobo Dioulasso. Specifically, sixteen (16) samples of refined cottonseed oils were collected : eight (8) samples taken in Ouagadougou-Pabré and eight (8) samples taken in Bobo Dioulasso. For peanut oils, sixteen (16) samples including eight (8) samples taken in Ouagadougou-Saaba and eight (8) samples taken in Bobo Dioulasso were collected. The Figure 1 gives the photo of samples of oils.

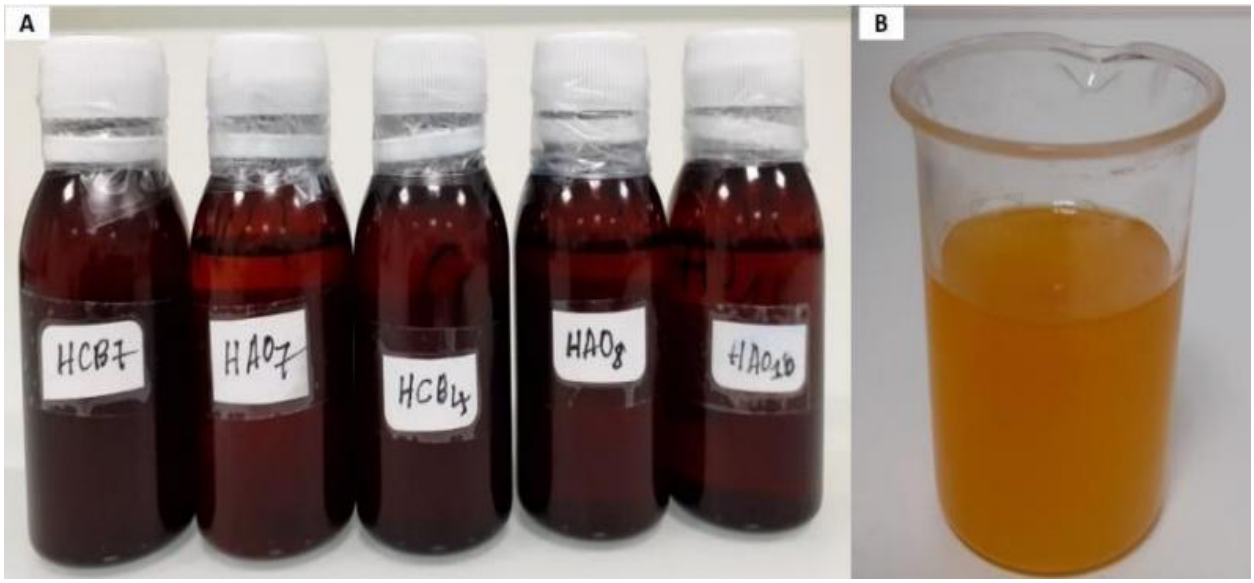


Figure 1. Oil samples after opening for analysis; A: HCB: Cottonseed oil collected in Bobo Dioulasso; HAO: Peanut oil collected in Ouagadougou; Numbers 4, 7, 8 and 10 are the samples numbers. B: Crude peanut oil in a beaker.

2.3. HPLC determination of α-tocopherol

2.3.1. α-tocopherol extraction

The α-tocopherol was quantified by HPLC according to the method used by Bele *et al.*[15] with some modifications. An Agilent 1200 series HPLC system/Agilent technology (Germany) was used. A C8 Kinetex 2.6 µm 100 Å 150*4.6 mm column (Phenomenex) and a DAD (Diode Array Detector) were used. The injection volume was 5 µL and a flow rate of 1 mL/min. The mobile phase consisted of solvent A 60% of methanol/ammonium acetate mixture 1 M (70:30, V/V) and solvent B 40% of methanol. The α-tocopherol wavelength detection is 295 nm. A quantity of 250 mg oil was weighed into a test tube. A volume of 5 mL mixture methanol/tetrahydrofuran (50:50, V/V) was added and vortexed (Stuart, UK) for one minute. The resulting solution was filtered through a 0.45 µm PTFE filter in a vial and injected for quantification.

2.3.2. Quantitative analysis

The identification and quantification of α-tocopherol in oil are based on the comparison of retention times with those of standards, linear regression of areas and concentrations of calibration points by the method of external standards. The α-tocopherol stock solution was prepared by dissolving 52.56 mg of α-tocopherol (Sigma Aldrich) with 20 mL tetrahydrofuran (Fisher scientific) and homogenizing. This solution was diluted to 0.1 mg/mL. Also, eight standards solutions were prepared with the methanol/tetrahydrofuran mixture (50:50, V/V) in 10 mL volumetric flasks. The standard solutions concentrations ranged from 0.002 mg/mL to 0.036 mg/mL. These solutions were filtered and injected in HPLC and processed under the same conditions as the oil samples. The α-tocopherol quantification was obtained using the calibration curve of the standards solutions areas as a function of concentrations.

2.4. Total phenolic compounds determination

2.4.1. Total phenolic compound extraction

Phenolic compounds were extracted adapted from the method used by Merouane *et al.* [16]. Three grams of oil were weighed in centrifuge vials. A volume of 3 mL methanol-water solvent (80:20, V/V) were added. After one minute of agitation (Stuart, UK), the solution was centrifuged (MR22i centrifuge/Jouan, France) at 6500 rpm for 10 minutes at 15 °C. The upper phase was collected. The lower phase underwent two additional extractions. The three supernatants were collected and dry evaporated under nitrogen using a puriVac-6™ at 30 °C. The dry residue was collected in 1.5 mL of the methanol-water mixture (80:20 ; V/V), filtered through a 0.45 µm PTFE filter and stored at −80 °C for analysis [16].

2.4.2. Total phenolic compounds determination

Oil samples containing total phenolic compounds, treated with the Folin Ciocalteu reagent in an alkaline medium (Sodium carbonate), develop a blue coloration measurable by spectrophotometry.

The coloration development is influenced by temperature and reaction time [17]. The total phenolic compounds quantification was performed by Folin Ciocalteu method using a gallery (Thermo scientific, Finland). 25 µL of oil extract was mixed with 125 µL of Folin Ciocalteu reagent (AppliChem, Germany) and diluted at 1/10. Finally, a volume of 100 µL sodium carbonate solution 7.5% (Sigma Aldrich, Germany) was added. The mixture was stirred and incubated at 30 ℃ for 30 min before reading the absorbance at 750 nm. The spectrophotometric analysis were repeated three times on each type of oil sample [18]. At the same time, a gallic acid solution (99% purity, Sigma Aldrich, China) was prepared. Each standard dilution solution was treated as the oil sample. Concentrations ranged from 0.0025 to 0.22 mg/mL. The total phenolic contents were calculated on the basis of the calibration curve of gallic acid and expressed as gallic acid equivalents.

2.5. DPPH radical scavenging activity determination

The method adapted on a gallery sequential analyzer (Thermo scientific, Finland) was used for the determination of the 2, 2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging activity. DPPH is a relatively stable free radical [19]. A discoloration from violet to colorless explains the power of the extract to trap this radical, which can be detected by a UV spectrophotometer. This discoloration highlights the antioxidant power of a sample by its capacity to trap the free radical [20]. Percent inhibition and antioxidant activities were obtained after determination of absorbance using the DPPH radical. The method consisted of spectrophotometric measurement of the intensity of the color change in solution depending on the amount of 2, 2-diphenyl-1-picrylhydrazyl (DPPH). The reaction was carried out by adding 12 µL of oil extract to 230 µL of DPPH (63.4 µM) solution. After incubation at 30 ℃ for 30 minutes, absorbance was read at 520 nm [21]. A control solution was performed by replacing DPPH with pure methanol. The scavenger activity/percentage inhibition of the DPPH radical of oil extract was calculated as follows [22]:

$$\%\text{DPPH} = \frac{\text{Control Absorbance} - \text{Sample Absorbance}}{\text{Control Absorbance}} \times 100 \quad (1)$$

The antioxidant properties are due to their ability to form an intramolecular hydrogen bond [23]. The standard solutions of trolox® were prepared and the concentration were ranged between 0.048 and 0.016 mg/mL. The oil antioxidant activities were calculated from the calibration curve obtained from a trolox® solution used as a reference and expressed in mg/mL of trolox equivalent in the oil extract.

2.6. Data analysis

The statistical analysis were performed using Excel 2013 and SPSS Version 20 software. Data analyses were replicated three times by oil samples. The Fisher test was used to compare the different values obtained at probability thresholds of p = 5% (significant if $p < 0.05$ and non-significant if $p > 0.05$).

3. Results and discussion

Chromatograms of oil samples and standard allowed the quantification of alpha tocopherol. A slight difference in retention time of alpha tocopherol peak is observed. This difference could be due to the mobile phase preparation. Also, an increase of the temperature especially that of the column decreases the retention of the solute. The α-tocopherol levels range from 4.79 to 16.49 mg/100 g for crude peanut oils. For refined cottonseed oils, the values range from 42.38 to 68.63 mg/100 g. Overall, the averages are 10.89 and 56.44 mg/100 g for crude peanut oils and refined cottonseed oil, respectively ($p < 0.05$). The α-tocopherol values of cottonseed oils are higher than those obtained for crude peanut oils. This difference could be related to the manufacturing process of peanut oils which are subjected to a high drying temperature during the manufacturing process of "*Koura-Koura*" which is a cake sold on the markets. Also, the exposure of the oils to the air is responsible for oxidation which decreases the α-tocopherol content as the loss of vitamin E is mainly due to oxidation reactions. The results for α-tocopherol and total phenolic compounds values are shown in Table 1.

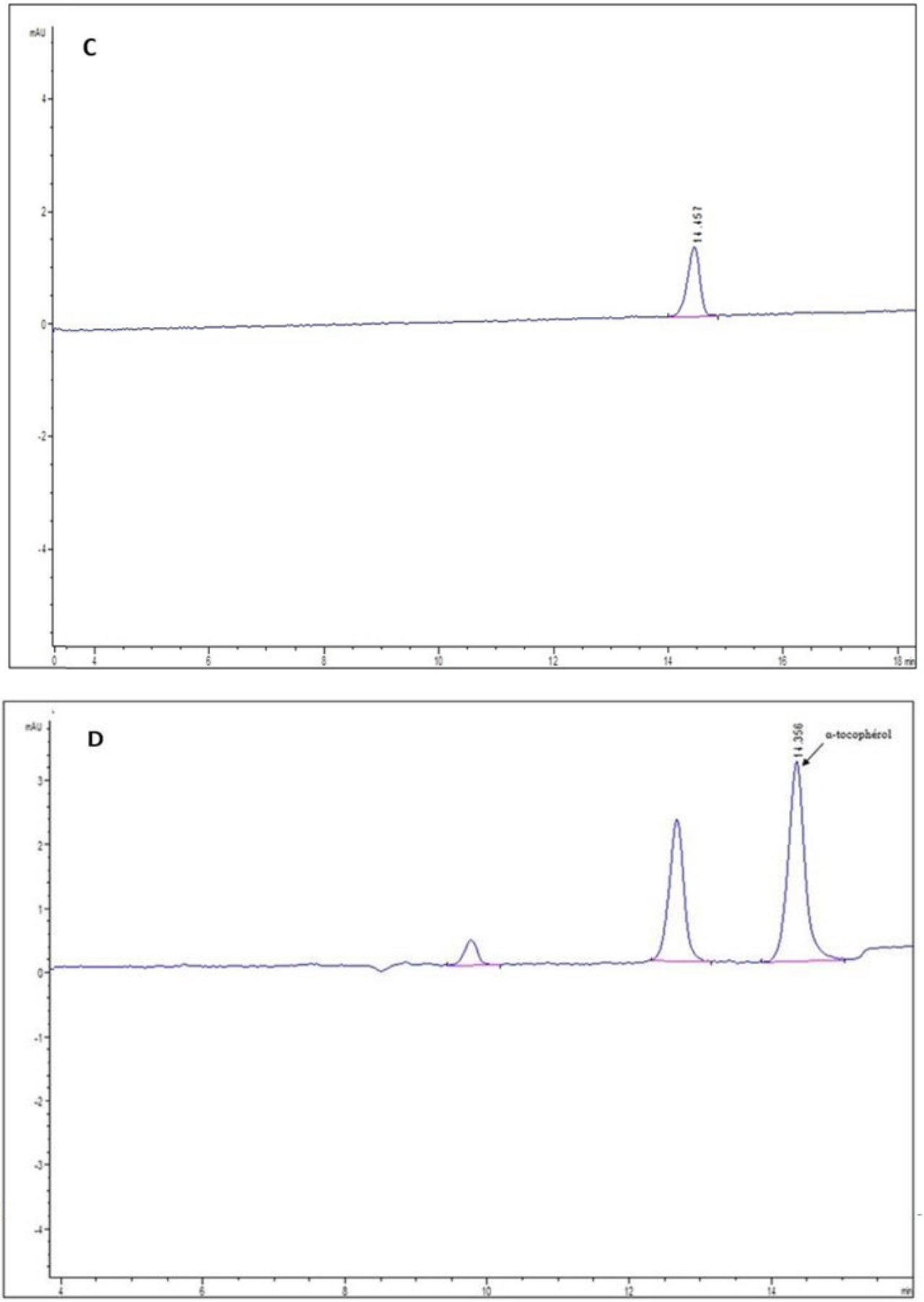
Table 1. Total phenolic compounds and α-tocopherol content.

Oil	City	Parameter			
		Range		Average ± SD	
		α–T (mg/100 g)	CPT (mg/100 g)	α-T (mg/100 g)	CPT (mg/100 g)
Peanut	OS	4.79–14.18	0.94–2.08	9.25 ± 3.20[a]	1.51 ± 0.40[a]
	BD	10.54–16.49	2.10–7.36	12.53 ± 1.89[a]	4.31 ± 1.94[a]
Cottonseed	OP	42.38–62.62	0.43–1.27	52.12 ± 8.72[b]	0.69 ± 0.26[b]
	BD	50.10–68.63	0.42–0.70	60.76 ± 7.30[c]	0.58 ± 0.11[c]
Peanut	OS & BD	4.79–16.49	0.94–1.36	10.89 ± 3.05[a]	2.91 ± 1.98[a]
Cottonseed	OP & BD	42.38–68.63	0.42–1.27	56.44 ± 8.96[a]	0.64 ± 0.20[a]

SD: Standard deviation; OS: Ouagadougou-Saaba; BD: Bobo Dioulasso; OP: Ouagadougou-Pabré; α-T: α-tocopherol; CPT: Total phenolic compounds. Numbers with different letters in the same column and by oil type do not show a significant difference at the 5% threshold.

A partial α-tocopherol degradation in oil is observed during the heating. The loss of α-tocopherol depend on the nature of oil and can exceed 50% [24]. Oil refining reduces α-tocopherol content by 15 to 20% during deodorization and especially during discoloration step [25]. The storage process leads to a decrease the tocopherol content of the seeds [26]. A partial degradation of tocopherols of 10 to 40% of their initial value after one to two hours of heating at 180 ℃ has been observed [25]. This may justify the low α-tocopherol levels of crude peanut oils because these oils are produced traditionally. Compared to other studies, the crude peanut oils value is higher than 100.16 mg/Kg [7], 102 mg/Kg [27] and lower than 12.66 mg/100 g [28]. For cottonseed oils, our average is above 28.62 mg/100 g [28]. In addition to their vitamin and therapeutic role [29], tocopherols are chain-breaking antioxidants of radical chains in membranes and foods [30]. The antioxidant activity is due to the 6-OH-chroman nucleus [25].

The Figure 2 gives the chromatograms of α-tocopherol of cottonseed oils and peanut oils.


C
mAU
11.457
min
D
mAU
11.356
α-tocophérol
min

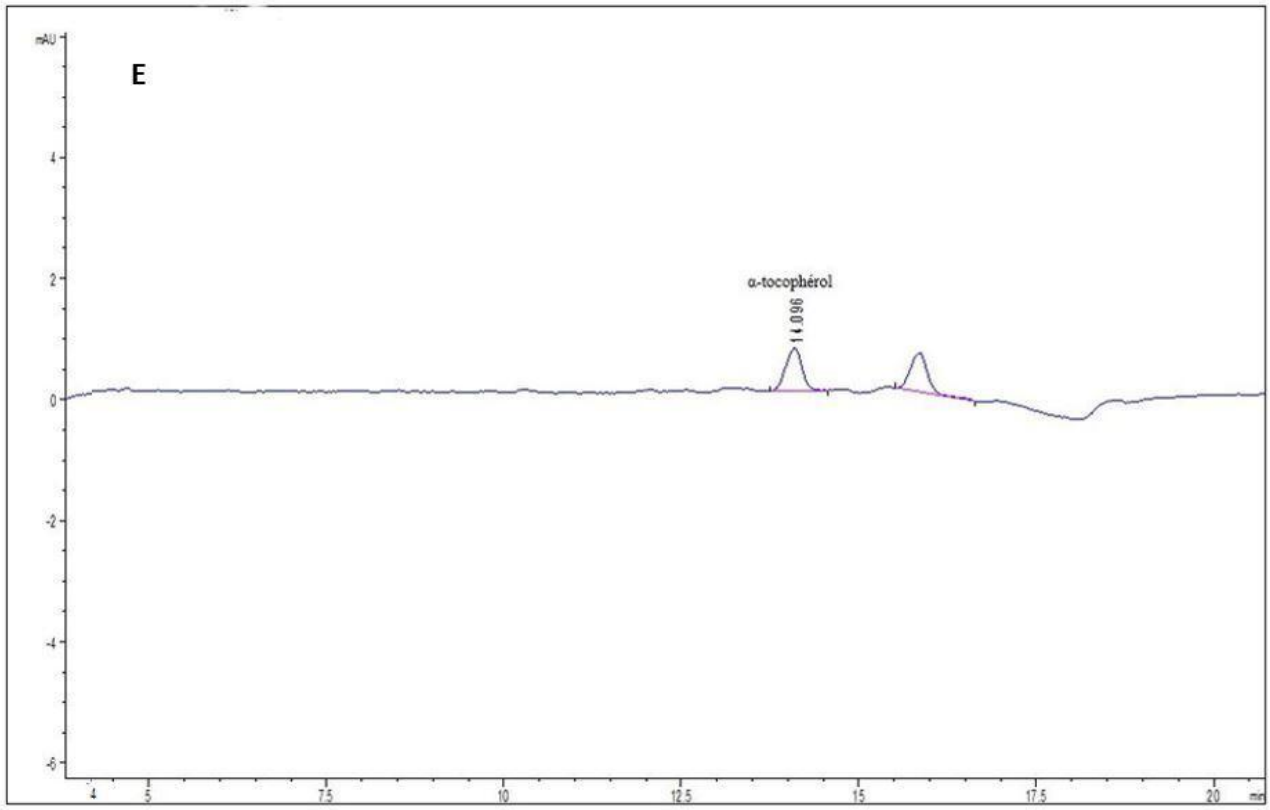


Figure 2. Chromatograms of α-tocopherol of cottonseed oils and peanut oils. C: α-tocopherol standards; D: Chromatogram of α-tocopherol of a cottonseed oil sample; E: Chromatogram of α-tocopherol of a peanut oil sample. Using the chromatogram of the standards, the different α-tocopherol was identified on the basis of retention times.

Total phenolic compounds values for peanut oils range from 0.94 to 7.36 mg gallic acid/100 g. For cottonseed oils, the values range from 0.42 to 1.27 mg gallic acid/100 g. Total phenolic compounds in this study for both types of oils are 0.64 and 2.91 mg gallic acid/100 g for refined cottonseed oils and crude peanut oils, respectively ($p < 0.05$). Total phenolic compounds in peanut oils are higher than those in cottonseed oils. This result is confirmed by several authors including Shad *et al*. [31] who found total phenolic compounds contents between 45.6 and 47.74 mg/100g for peanut oils. For cottonseed oils, Mohdaly *et al*. [32] obtained 9.87 mg/100 g compared to 8.22 mg/g [33]. All values are higher than the values obtained in this study. The low value of phenolic compounds in our study could be due to extraction processes and oils oxidation. A large part of these compounds are eliminated due to oil extraction methods and conditions encountered during refining including high temperatures, acid and alkaline agents, and metal equipment [34]. Phenolic compounds or "bio-phenols" [29] are effective antioxidants with antimicrobial, lipid-lowering, cholesterol-lowering and anti-carcinogenic properties [35]. The redox properties of phenolic compounds allow them to act as reducing agents, hydrogen donors and singlet oxygen scavengers [36]. Phenolic compounds of natural origin are characterized by a high antioxidant capacity comparable to tert-butylhydroquinone (TBHQ), which is synthetic, and can replace it in the food industry [37].

The inhibition percentages of peanut oils produced range from 5.96% to 43.67%. For refined cottonseed oils, the values range from 2.37 to 12.90 %. The average inhibition percentages of crude

peanut oils and refined cottonseed oils in this study are 17.97% and 5.58%, respectively. The inhibition percentages of DPPH radical of peanut oils are higher than those of cottonseed oils ($p < 0.05$). The DPPH method can predict the oxidative stability of oils by taking into account the initial concentration of free radical scavengers and the oxidation time required for the consumption of the first antioxidants [38]. Among the lipid radicals formed during auto-oxidation that are alkoxyl (RO), peroxyl (ROO) and alkyl (R) radicals, peroxyl lipid radicals (ROO). This compounds are the major targets for hydrogen-donating antioxidants [38]. The results of inhibition percentages and antioxidant activities of cottonseed oils and peanut oils analyzed are presented in Table 2.

Table 2. Antioxidant activity and inhibition percent of DPPH radical.

Oil	City	Parameter			
		Range		Average ± SD	
		IH (%)	AO (mg/100 g)	IH (%)	AO (mg/100 g)
Peanut	OS	5.96–16.60	0.32–0.77	10.24 ± 3.91[a]	0.50 ± 0.16[a]
	BD	14.89–43.67	0.68–1.81	25.71 ± 11.19[a]	1.12 ± 0.44[a]
Cottonseed	OP	3.54–12.90	0.20–0.60	6.33 ± 2.81[b]	0.32 ± 0.12[b]
	BD	2.37–7.71	0.11–0.39	4.83 ± 1.49[c]	0.21 ± 0.10[c]
Peanut	OS & BD	5.96–43.67	0.32–1.81	17.97 ±11.37[a]	0.81 ± 0.45[a]
Cottonseed	OP & BD	2.37–12.90	0.11–0.60	5.58 ± 2.44[a]	0.27 ± 0.12[a]

SD: Standard deviation; OS: Ouagadougou-Saaba; BD: Bobo Dioulasso; OP: Ouagadougou-Pabré; IH: inhibition; AO: Antioxidant activity. Numbers with different letters in the same column and by oil type do not show a significant difference at the 5% threshold.

DPPH has been commonly used for the screening of phenolic compounds with high free radical scavenging capacity [39]. High free radical scavenging capacity is considered to have strong antioxidant activity. The DPPH method has been used as one of the basic screening steps in the search for novel antioxidant compounds in organic solvent extracts from natural resources. This method is specifically designed for the determination of hydrogen-donating antioxidant compounds [38]. The 2, 2-diphenyl-1-picrylhydrazyl (DPPH) method can be used to compare the oxidation stability of edible oils from different sources or process manufactures [38].

Antioxidant activities range from 0.32 to 1.81 mg/100 g trolox equivalent for peanut oils and 0.11 to 0.60 mg/100 g trolox equivalent for cottonseed oils. Overall, the average antioxidants activities are 0.81 and 0.27 mg/100 g trolox equivalent for peanut oils and cottonseed oils, respectively ($p < 0.05$). As a result, peanut oils were more stable than cottonseed oils. This stability has been proven. Peanut oil is very stable and resists well to cooking, even at high temperatures. The stability of peanut oil is due to the fatty acid composition dominated by monounsaturated fatty acid more than 47% [40] while cottonseed oil is rich in linoleic acid (33–58%). This composition justifies the low stability of cottonseed oils compared to peanut oils.

4. Conclusion

This study assessed the antioxidant levels and antioxidant activity of vegetable oils produced in Burkina Faso. Refined cottonseed oils have the highest levels of α-tocopherol with an average of 56.44 mg/100 g. Peanut oils have the highest levels of total phenolic compounds with 2.91 mg/100 g

of gallic acid equivalent. The inhibition percentage average of peanut oils is 17.97% and the antioxidant activity average is 0.81 mg/100 g. The inhibition percentages and the antioxidant activity values of peanut oils are higher than those of cottonseed oils. Thus, cottonseed oils have the highest vitamin potency while peanut oils have significant antioxidant activity. The oils produced in Burkina Faso are a source of biomolecules, particularly α-tocopherol. Their consumption is nutritionally beneficial for the consumer's health. However, good manufacturing and conservation practices are essential for the preservation of these biomolecules in vegetable oils. Also, it is essential to control the temperature and limit the exposure of the oils to oxygen and light during production and storage. The results could be used for future recommendations about human feeding programs in Burkina Faso.

Conflict of Interest

The authors declare no conflicts of interest.

Acknowledgments

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