Cholesterol Levels in Vegetable Oils Produced in Burkina Faso

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Abstract   Vegetable oils are widely produced and consumed in Burkina Faso. The objective of this work is to evaluate the cholesterol level, refractive value and saponification value of crude peanut oils and refined cottonseeds oils produced in Burkina Faso. The study was carried out on 61 samples of refined cottonseeds oils and crude peanut oils collected in Ouagadougou, Bobo Dioulasso and surrounding areas. Cholesterol level was evaluated by HPLC, refractive and saponification values were determined by physico-chemical standard methods. The results show that 64.52% of the saponification value of peanut oils fall within the compliance range of the *Codex Alimentarius* standard compared to 63.33% for cottonseeds oils. The average saponification values are respectively 192.06 mg KOH/g and 194.16 mg KOH/g for crude peanut oils and refined cottonseeds oils ($p>0.05$). All cottonseeds oils have refractive value in accordance with the *Codex Alimentarius* standard while 90.32% of peanut oils have refractive value in accordance with the standard. The average refractive value are 1.468 and 1.471 for crude peanut oils and refined cottonseeds oils respectively ($p<0.05$). The majority of the refractive value and saponification value show an acceptable level in terms of oils for food consumption. Cholesterol was detected in 20% and 38.70% of the peanut oils and cottonseeds oils samples analyzed respectively. The cholesterol averages are 0.64 and 2.49 mg/100g for crude peanut oils and refined cottonseeds oils respectively ($p>0.05$). Almost all cholesterol values of different oil samples are lower to the *Codex Alimentarius* standard.

Cite This Article: Zio Souleymane, Zongo Oumarou, Ouattara Ismaël, Bazié Raoul Sylvain Bazoin, Somda Namwin Siourimè, Traoré Korotimi, Nikiéma Fulbert, Elothmani Driss, Traoré Yves, and Savadogo Aly, "Cholesterol Levels in Vegetable Oils Produced in Burkina Faso." *American Journal of Food Science and Technology*, vol. 8, no. 4 (2020): 147-153. doi: 10.12691/ajfst-8-4-3.

1. Introduction

All seeds, fruits and almonds contain oils, but only those that are used to produce oils industrially and are grown for this purpose are called oilseeds [1]. Oils from these seeds have several functions. Lipids are involved in the structure of foods, their sensory characteristics and their microbiological preservation. Vegetable oils are means of heat transfer during cooking, coating and release agents or as carriers of lipophilic flavors and dyes [2]. In view of the nutritional and economic importance, the large price difference between vegetable oils can lead to the temptation to falsify by introducing all or a lower cost oils part into a higher priced oils [3]. Then, there are laboratory methods used to detect oils adulteration. These methods include the determination of methyl esters [4], sterols content [5], tocopherols and tocotrienols levels

which are identity factors for vegetable oils [6] and triglyceride analysis [3]. Plant sterols, called phytosterols are minor compounds present in all vegetable oils with an average ranged from 0.1 to 0.5% [6]. The sterols of animal origin are called zoo sterols. Among them, cholesterol is the main vertebrate sterol provided by meat, dairy products and eggs [8]. These biomolecules are both effective in the field of revitalization and anti-free radical protection of the epidermis and in relaunching cell activity [9]. Cholesterol, longer considered to be exclusively animal, has been demonstrated in many plants [10,11]. Several authors have detected the presence of cholesterol in vegetable oils. In addition to its beneficial role for the body, low density lipoproteins cholesterol, cholesterol transported in low density lipoproteins, have a key role in the initiation and development of atherosclerosis. It corresponds to the atherogenic fraction of cholesterol, as it tends to accumulate in the arterial wall and oxidize [11]. Atherosclerosis is the major cause of cardiovascular

disease and its clinical complications (cerebrovascular accident, myocardial infarction) [12]. As a result, international standards have established maximum limits for cholesterol in edible oils not to be exceeded [3]. Other parameters such as refractive value and relative density are physical measures of the vegetable oils adulteration, as different oils have a characteristic density and refractive value [13]. The refractive and saponification values allow to control oil purity. Hence, the purpose of this study was to assess the cholesterol level, saponification value and refractive value of crude peanut oils and refined cottonseeds oils produced and consumed in Burkina Faso.

2. Material and Methods

2.1. Samples Collection

The oil samples collected were crude peanut oils and refined cottonseeds oils. A total of sixty-one (61) samples were collected in 500 ml amber vials and sealed, coded, transferred to the laboratory and stored in icebox for analysis. This work was carried out at the National Public Health Laboratory of Burkina Faso. Crude peanut oils were collected from production sites and some markets while refined cottonseeds oils were collected from production factories based in the industrial zone of Kossodo in Ouagadougou-Pabré and Bobo Dioulasso. Specifically, thirty (30) samples of refined cottonseeds oils including (15) samples taken in Ouagadougou-Pabré and 15 samples taken in Bobo Dioulasso were collected. For peanut oils, 31 samples including 16 samples from Ouagadougou-Saaba and 15 samples taken in Bobo Dioulasso were collected.

2.2. Refractive Values Determination

The refractive values must be determined on the perfectly anhydrous and filtered oil. A drop of oil was placed on the tank of the apparatus and the refractive value was given by the Mettler Toledo refractometer (MSC40A41) at 20°C [14].

2.3. Saponification Values Determination

A quantity of 2 g oil sample was weighed into a conical flask. The 2 g test portion was determined on the basis of a saponification number of 170 to 200. A volume of 25 mL an ethanolic solution of potassium hydroxide and a few boiling regularisers were added to the test portion. The reflux condenser was connected to the flask on the heater. The mixture was boiled gently, stirring occasionally, for 60 min. To this hot solution was added 0.5 to 1 mL of phenolphthalein solution and titration with the hydrogen chloride (0.5 M) standard solution was carried out until the pink color of the indicator disappeared. A blank test was carried out following the same procedure and also using 25 mL of potassium hydroxide ethanolic solution but omitting the test sample [14]. The saponification value was calculated by the following formula: $SV = (Vo - V1) \times C \times 56.1/PE$ at mg KOH/ g where Vo

= volume of the hydrogen chloride standard solution (mL); V1 = volume of the hydrogen chloride standard solution used for the determination; PE = mass of the test sample (g) and C = concentration of the hydrogen chloride standard solution (mol/L).

2.4. Cholesterol Level Determination

The cholesterol assessment in edible oils was conducted according to the method used by Almeida *et al*. [16]. The oils were saponified as follows: 2 g of oil samples were weighed into a test tube. A volume of 4 mL of potassium hydroxide solution 50% and 4 mL of absolute ethanol were added. The mixture was placed in a water bath at 60°C for 10 min for complete solubilization. Then a volume of 5 mL of distilled water was added and the mixture was cooled. The unsaponifiable fraction was extracted three times with 10 mL of hexane. An aliquot of 3 mL of the hexanolic extract was dried under nitrogen flow. The dry residue was dissolved in a 3 mL of the mobile phase consisting of acetonitrile and isopropanol (70:30 v/v)) and transferred into vials for HPLC analysis. An Agilent Technologies 1100 series liquid chromatography system equipped with a UV-visible detector, the wavelength was fixed at 208 nm and the separation was carried out with a 150 mm x 4.6 mm 5 µm particle size ODS Hypersil analytical column. The injection volume was fixed at 20 µL with a constant flow rate of 1 mL/min. The identification and quantification of cholesterol in oils 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 [15].

2.5. Statistical Analysis

The statistical analysis was performed using Excel 2013 and SPSS Version 20 software. 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

3.1. Refractive Values of Vegetable Oils

The refractive values of the oil samples analyzed are shown in Table 1. The values range from 1.469 to 1.470 with an average of 1.469 for crude peanut oils produced in Ouagadougou-Saaba. The refractive values of peanut oils produced in Bobo Dioulasso range from 1.460 to 1.470 with an average of 1.467. The refractive averages variation of crude peanut oils is significant ($p<0.05$). For refined cottonseeds oils, the values range from 1.471 to 1.472 with an average of 1.471 for those oils produced in Ouagadougou-Pabré, while those produced in Bobo Dioulasso, have values ranging from 1.471 to 1.473 with a mean of 1.471. The variation in the averages for refined cottonseeds oils is non-significant ($p>0.05$). Overall, the averages are respectively 1.468 and 1.471 for crude peanut oils and refined cottonseeds oils with a significant variation ($p<0.05$).

3.2. Saponification Values of Vegetable Oils

The saponification values of the oils analyzed are presented in Table 1. The values range from 165.49 to 196.25 mg KOH/g for crude peanut oils produced in Ouagadougou-Saaba with an average of 187.03 mg KOH/g. The oils produced in Bobo Dioulasso have saponification values ranging from 185.03 to 208.65 mg KOH/g with an average of 197.43 mg KOH/g. The variation between the averages is significant ($p<0.05$). For refined cottonseeds oils, the saponification values range from 189.14 to 208.65 mg KOH/g with an average of 195.86 mg KOH/g for those produced in Ouagadougou-

Pabré. The saponification values range from 168.13 to 203.15 mg KOH/g with an average of 192.45 mg KOH/g for cottonseeds oils produced in Bobo Dioulasso. The variation in the averages of cottonseeds oils in the two cities and surrounding areas is non-significant ($p>0.05$). The averages are respectively 192.06 mg KOH/g and 194.16 mg KOH/g for crude peanut oils and refined cottonseeds oils with no significant variation ($p>0.05$).

3.3. Cholesterol Level Assessment

The cholesterol contents of cottonseeds oils and peanut oils analyzed are presented in Table 2.

Table 1. Saponification and refractive values average of analyzed oil by type and sampling City

Oils type	Samples codes	Saponification value (mg KOH/g)		Refractive value	
		Range	Average and standard deviation	Range	Average
Peanut oils	OS	165.49-196.25	$187.03^a \pm 9.60$	1.469-1.470	$1.469^a \pm 0.000$
	BD	185.03-208.65	$197.43^b \pm 7.25$	1.460-1.470	$1.467^a \pm 0.003$
Cottonseeds oils	OP	189.14-208.65	$195.86^a \pm 5.04$	1.471-1.472	$1.471^a \pm 0.000$
	BD	168.13-203.15	$192.45^a \pm 10.61$	1.471-1.473	$1.471^a \pm 0.000$
Peanut oils	OS and BD	165.49-208.65	$192.06^{cd} \pm 9.93$	1.460-1.470	$1.468^a \pm 0.002$
Cottonseeds oils	OP and BD	168.13-208.65	$194.16^{ef} \pm 8.34$	1.471-1.473	$1.471^b \pm 0.000$

Value of p (significant if $p> 0.05$ and non-significant if $p> 0.05$); a, b, c, d, e, f: the values which have the same letter in the same column and by oils type, do not present a significant difference according to the Fisher test at the 5% threshold; OS: Ouagadougou-Saaba; BD: Bobo Dioulasso and OP: Ouagadougou-Pabré.

Table 2. Cholesterol range and averages of analyzed oils by type and sampling City

Oils type	Samples codes	Sample quantity	Range (mg/100g)	Average (mg/100g)	F	*p*-Value
Peanut oils	OS	16	ND-2.31	1.00 ± 0.85	7.19	0.01
	BD	15	ND-2.09	0.25 ± 0.68		
Cottonseeds oils	OP	15	ND-43.37	4.99 ± 12.01	2.58	0.11
	BD	15	ND	ND		
Peanut oils	OS and BD	31	ND-2.31	0.64 ± 0.85	1.38	0.24
Cottonseed oils	OP and BD	30	ND-43.37	2.49 ± 8.72		

ND: No detected considered as zero; p values (significant if $p < 0.05$ and not significant if $p > 0.05$); OS: Ouagadougou-Saaba; BD: Bobo Dioulasso and OP: Ouagadougou-Pabré.

Figure 1. Compliance rate at percent with the *codex alimentarius* standard of refractive value, saponification value and cholesterol presence rate in oil samples analyzed
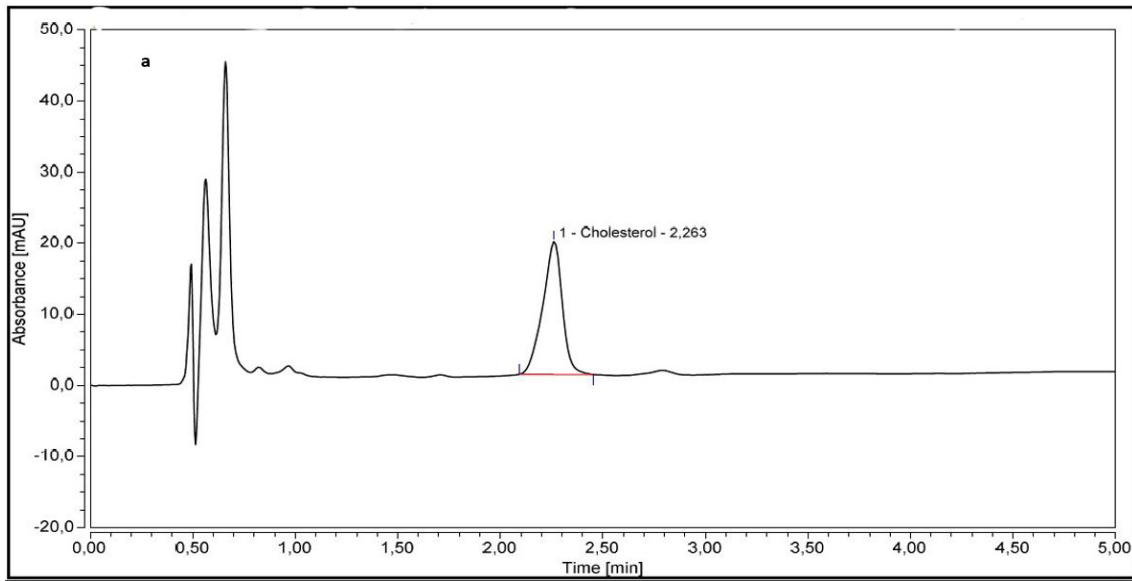
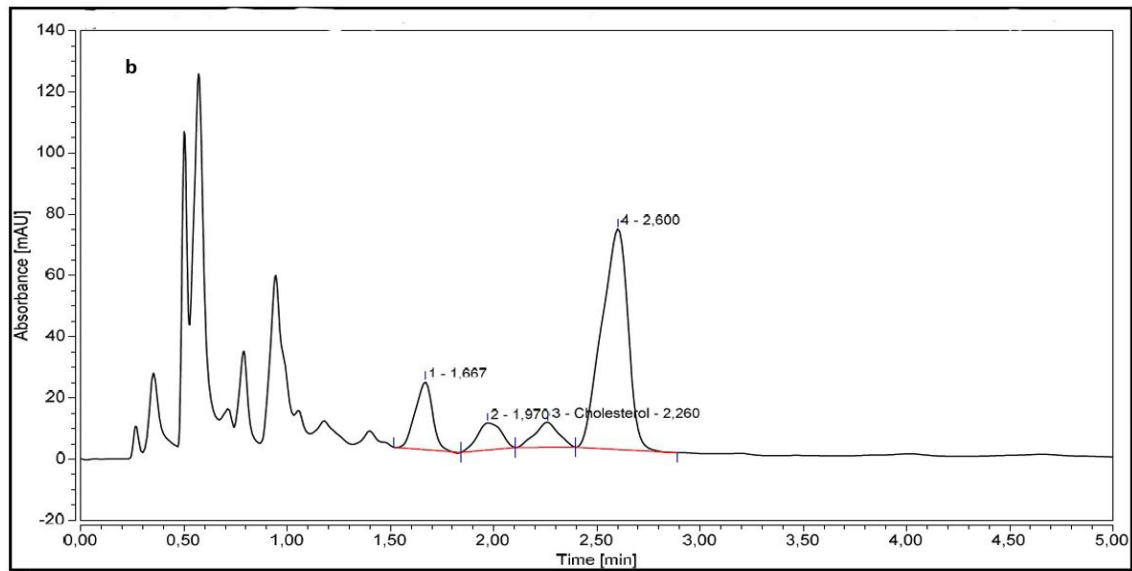**Figure 2.** (a)-Chromatogram of cholesterol standard.

Figure 3. (b)-Chromatogram of cottonseeds oil sample analyzed. Cholesterol is identified by peak number 3.
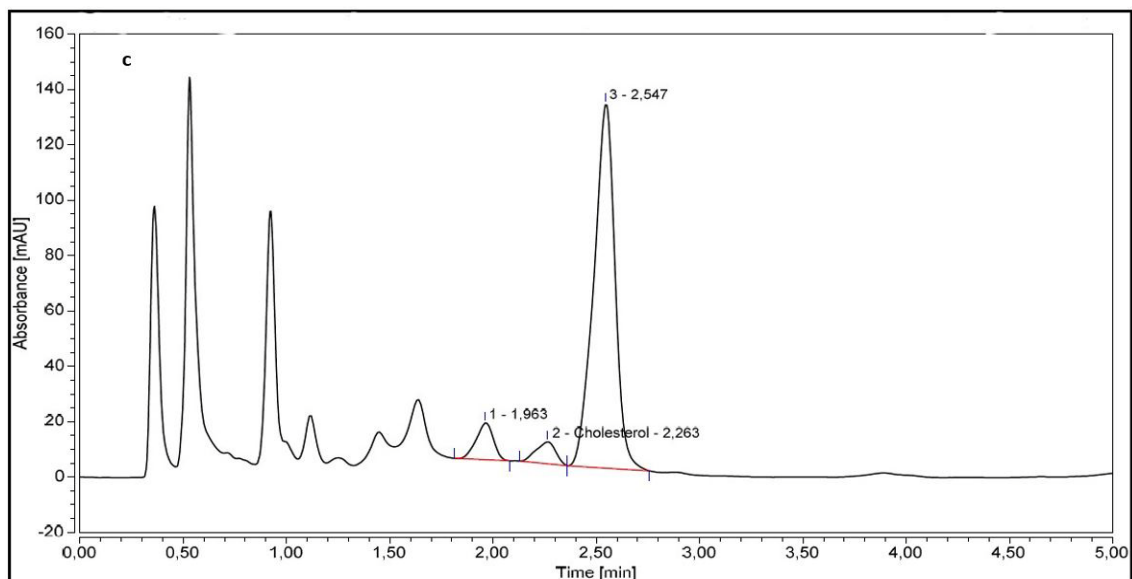

Figure 4. (c)-Chromatogram of peanut oil sample analyzed. Cholesterol is identified by peak number 2

The cholesterol level in crude peanut oils and refined cottonseeds oils has been investigated. Cholesterol was detected in 62.50% samples of crude peanut oils produced in Ouagadougou-Saaba with contents ranging from 0 to 2.31 mg/100g with an average of 1.00 mg/100g. The cholesterol contents of peanut oils produced in Bobo Dioulasso vary from 0 to 2.09 mg/100g with a presence rate of 13.33%, for an average 0.25 mg/100g. The cholesterol contents average of crude peanut oils is statistically significant ($p<0.05$). For refined cottonseeds oils produced in Ouagadougou-Pabré, the cholesterol contents ranged from 0 to 43.37 mg/100g, with an average 4.99 mg/100g and a presence rate is 40%. Cholesterol has not been detected in refined cottonseeds oils produced in Bobo Dioulasso. The cottonseeds oils averages variation is non-significant ($p>0.05$). In oil samples analyzed, cholesterol was detected in 20% and 38.70% of the peanut oils and cottonseeds oils samples respectively. In general, the cholesterol averages are respectively 0.64 and 2.49 mg/100g for crude peanut oils and refined cottonseeds oils ($p>0.05$). Figure 1 shows the compliance rates of refractive and saponification value of oil samples with the *Codex Alimentarius* standard and cholesterol presence rate in oil samples analyzed.

Quantification chromatograms are given in Figure 2, Figure 3 and Figure 4. A qualitative investigation for the detection and confirmation of the HPLC method used was carried out.

4. Discussion

4.1. Refractive Values

The refractive values of crude peanut oils produced in Ouagadougou-Saaba are within the range allowed by the *Codex Alimentarius* (1.468-1.472) except for some samples of peanut oils produced in Bobo Dioulasso which are below the standard. This would justify the presence of free fatty acid resulting from the hydrolysis of triglycerides. The presence of free fatty acid lowers the refractive values of vegetable oils [16]. Also, the presence of impurities is an inferiority factor of the refractive values and this is justified by the no refining process of crude peanut oils. For cottonseeds oils, all refractive values are in accordance with the *Codex Alimentarius* standard allowed for cottonseeds oils (1.470-1.473). This conformity of cottonseeds oils is justified by the refining process that removes impurities including free fatty acids present. The sample rate of peanut oils analyzed in this study with refractive values conforming to the *Codex alimentarius* standard is 90.32% while all samples of refined cottonseeds oils have values conforming to the standard. Compared to other studies, the values obtained in this study are lower than the 1.471 and 1.472 of crude peanut oils and refined cottonseeds oils, respectively, obtained by Zio *et al*. [14]. The value of 1.468 for peanut oils in this study is higher than the 1.467 and 1.463 values found by Kandji [17] and Chabiri *et al*. [13], respectively. These low values obtained by the authors could be related to the presence of impurities or free fatty acids. Diatta [18] and Bathily [19] reported an identical value of 1.468 in peanut oils. Refractive value is an important criterion for

the identification and oils purity. On this basis, oils rich in oleic acid have a refractive value between 1.468 and 1.472 which is consistent with peanut oils very rich in oleic acid. Oils rich in linoleic acid which have a refractive values between 1.471 and 1.477 which is consistent with the cottonseeds oils analyzed [20].

4.2. Saponification Values

The saponification values of peanut oils range from 165.49 to 208.65 mg KOH/g with an average of 192.06 mg KOH/g. Some values are outside the *Codex Alimentarius* standard for the saponification value of peanut oils (187-196 mg KOH/g) [6]. The average saponification value of peanut oils in this study is lower than the value of 194.12 mg KOH/g obtained by Zio *et al.* [14]. For cottonseeds oils, the saponification values range from 168.13 to 208.65 mg KOH/g with an average of 194.16 mg KOH/g. Some saponification values of cottonseeds oils are lower and others values higher than the *Codex Alimentarius* standard (189-198 mg KOH/g) but the average is still in line with the standards. The sample rate of peanut oils analyzed in this study with saponification values according to the *Codex alimentarius* standard is 64.52% compared to 63.33% for cottonseeds oils samples. The saponification value of cottonseeds oils reported in this study (194.16 mg KOH/g) is higher than those found by Zio *et al*. [14] which was 188.032 mg KOH/g, and lower than those obtained by Dimberu (228.55 mg KOH/g) [21]. The saponification value allows to control the purity of the oils but also its saponification aptitude. A higher saponification values indicates a high proportion of lower fatty acids since the saponification values is inversely proportional to the weight or length of the average molecular chain of fatty acids [21]. The saponification values reflects the length of the hydrocarbon chains of fatty acids [20]. Also, it indicates the deterioration level of oils. The saponification value increases with storage time because fatty acids are likely to be formed, which increases this values. This also indicates that these degraded oils stored for a long time can play a favorable role in the profitable production of soaps and toiletries [22].

4.3. Cholesterol Level

The cottonseeds oils and peanut oil samples analyzed contain cholesterol. This could mean that some analyzed oils are fraudulent because the sterol profile is used for the detection of adulterations [5]. Several authors have quantified cholesterol in various vegetable oils including refined cottonseeds oils and peanut oils. This is the case of the average 1.39 mg/ml obtained by Okpuzor *et al*. [23] determined by spectrophotometry, the average 0.20 mg/ml reported by HPLC for peanut oils, and the average 131.90 mg/l for cottonseeds oils [21]. All cottonseeds oils and peanut oils values those authors are higher than our averages for different oils analyzed. Also, the peanut oils average in this study is lower than the 1.6 mg/100g obtained by Mariod *et al*. [24]. The cottonseeds oils average 2.49 mg/100g for this study is lower than the average 3.20 mg/100g found by Mariod *et al*. [24]. According to Behrman and Venkat [25], cholesterol,

contrary to popular belief, is present in plants. The presence of cholesterol has been demonstrated in many plants [10,11]. In addition to these assertions, the presence of cholesterol in vegetable oils could be due to interference with other compounds with chemical structures similar to the sterols structure such as sterol methyls, triterpene alcohols, vitamin D and beta-carotene. According to Kamm *et al.* [26], the biosynthetic conversion of β-sitosterol during the deodorization process yields cholesterol. Cholesterol level may be increased during deodorization due to the steam treatment [26] due to the biosynthetic conversion. In addition, the presence of cholesterol in vegetable oils could be related to a mixture with animal fat. Although having an indispensable role in the body including essential elements to the membrane and serves as a precursor to the synthesis of bile acids, steroid hormones and vitamin D, cholesterol is involved in certain pathologies due to increased levels of low density lipoproteins [21]. Cholesterol is transported in the bloodstream by particles called lipoproteins. But too much circulating cholesterol can damage the arteries, especially the coronary arteries that supply the heart. This leads to the build-up of cholesterol-laden "plaques" in the walls of the vessels, a condition called atherosclerosis. When blood flow to the heart is impeded, the heart is hindered, the heart muscle is deprived of oxygen, causing chest pain. If a blood clot completely blocks a coronary artery, atherosclerosis can cause a heart attack (myocardial infarction) or death [27]. As a result, level in the human diet that should not be exceeded are allowed. The *Codex Alimentarius* has set cholesterol maximum level for vegetable oils. This is the case for cottonseeds oils and peanut oils, which are respectively 0.7 to 2.3% and 3.8% of total sterols [6]. It is necessary to limit cholesterol consumption to less than 300 mg per day [28]. The total or partial elimination of sterols whose cholesterol is made during the oils refining process. Indeed, sterols are eliminated with odorous substances such as free fatty acids, tocopherols, saturated and unsaturated hydrocarbons and many others during the deodorization process [29].

5. Conclusion

This study demonstrated the cholesterol presence in crude peanut oils and refined cottonseeds oils produced and consumed in Burkina Faso in the cities of Ouagadougou, Bobo Dioulasso and surrounding areas. Cholesterol was identified in 20% and 38.70% of the peanut oils and cottonseeds oils samples respectively. This has removed the doubt about the possible presence of cholesterol in vegetable oils, given its involvement in atherosclerosis. However, cholesterol is present in low levels compared to *Codex Alimentarius standard* or even absent in some oil samples. Thus, the consumption of vegetable oils produced in these cities may not pose a risk for consumers. As for the refractive values, they reveal the oils purity with the exception of a few samples of peanut oils which do not conform to the standard due the nature of crude oils. Refractive values rate of peanut oils conforming to the *Codex alimentarius* standard is 90.32% while all samples of refined cottonseeds oils have values conforming to the standard. The same applies to the

saponification values, which makes it possible to control the oils purity and its aptitude for saponification. Saponification values according to the *Codex alimentarius* standard is 64.52% for peanut oils and 63.33% for cottonseeds oils samples. Overall, the oils analyzed are conform to their identity and free of fraudulent character.

Acknowledgements

Conflict of Interest

The authors state that there are no conflicts of interest.

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