JCHR (2019) 9(4), 321-329

Journal of Chemical Health Risks

www.jchr.org

ORIGINAL ARTICLE

Comparative Study on Cholesterol Content and Physicochemical Properties of Some Branded and Unbranded Commercial Edible Oils in Khulna, Bangladesh

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(Received: 20 April 2019 Accepted: 7 September 2019)

KEYWORDS

Edible oils;
Cholesterol;
Iodine value;
Peroxide value;
Saponification value;
Bangladesh

ABSTRACT: Edible oils are an essential component for cooking. Presence of an elevated amount of cholesterol in edible oil might cause several diseases. Therefore, the present study aims to estimate the amount of cholesterol in fourteen branded (BVO) and unbranded (UVO) commercially available edible vegetable oils in Khulna, Bangladesh. Besides, to ensure the quality of the oils, iodine value, saponification value, acid value, insoluble impurities and peroxide value were determined. Quantitative analysis of cholesterol was performed by Liebermann-Burchard method and the result indicates UVO black cumin oil has highest (525.49 ± 0.67 mg/L) cholesterol content whereas BVO sunflower oil contains lowest cholesterol content (145.36 ± 0.73 mg/L). The ascending order of the cholesterol content in various vegetable oils is sunflower oil (BVO)<olive oil (BVO)<mustard oil (BVO)<olive oil (UVO)<palm oil (UVO)<soybean oil (BVO)<coconut oil (BVO)<sesame oil (BVO)<coconut oil (UVO)<soybean oil (UVO)<sesame oil (UVO)<mustard oil (UVO)<black cumin oil (BVO)<black cumin oil (UVO). A survey report was prepared by collecting edible oils consuming data among 300 families in Khulna region, Bangladesh. Majority of the people are consuming BVO soybean oil and UVO palm oil. This study reveals that there are no cholesterol-free oils in the market which is a matter of concern and health implication. Therefore, the vegetable oils in industries manufacturing and marketing are arising in confusion with labeling their products as "cholesterol-free" among the people. They should mention the quantity of cholesterol present in their vegetable oils.

INTRODUCTION

Plants are the main sources of food, medicine, shelter, clothing, and oil. Oil is yielded in many plants but generally, oil is collected from oilseed plants like soybean, groundnut, palm, and sunflower. Foods act as fuel for the human to keep our body fit. Among these foods, oil is a vital part of the human diet. It can supply sufficient energy for the people. Mainly lipid and triacylglycerol in oil are the major sources of energy. Moreover, oil is riches in unsaturated and saturated fatty acids like oleic, linoleic, linolenic, myristic, palmitic, stearic acid that can control different biochemical process in the human body [1-3]. Besides these, oil can contribute other biological molecules like cholesterol being a lipid molecule. It is naturally synthesized in animals [4]. Cholesterol can be classified as two types i.e. low-density lipoprotein (LDL) and high-density lipoprotein (HDL). Both types of cholesterols have different functions in human health mainly in the cardiac cell. LDL is known as bad cholesterol that is responsible for narrowing coronary arteries. As a result, one can suffer from coronary artery diseases [5]. At present, heart diseases are increasing at an alarming rate. The reason

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DOI: 10.22034/jchr.2019.669296

behind this is hypercholesterolemia. Cholesterol is not only always harmful to us but sometimes it is essential for vital biological functions like assisting as a precursor of vitamin D and bile acids. Besides, they act as a structural element of the cell wall [6]. A diet with LDL elevates the content of cholesterol in the blood. So, at present people is concern about this and they want to purchase oil that is free from cholesterol [7]. During the industrial processing of vegetable oil, the composition of fatty acid is changed that leads to the risk of cardiovascular diseases (CVD) [8, 9]. Chemical nature of the essential oil can be changed depending on the variety of conditions like climate change, the composition of the soil, time of collection and the stage of plant growth [10]. The quality of oil can be ensured by knowing some physicochemical parameters like acid value, saponification value, viscosity, peroxide value and iodine value [11, 12]. Iodine value estimates the amount of unsaturated fatty acids present in the oil. The oxidation i.e. stability of the oil is governed by the iodine value. Generally higher iodine value indicates that the oil is sensitive to oxidation and it can be easily attacked by oxidants. Moreover, acid and peroxide values are common indicators of fat oxidation [13]. Determination of saponification value not only helps to predict the chain length of fatty acid but also it measures the average molecular weight of fatty acids. If the saponification value is found as low that indicates the presence of long-chain fatty acids in oil [14]. Analysis of above parameters ensures the quality and suitability of edible oils. Therefore, the present study designed to estimate the level of cholesterol and other physiochemical parameters in most popular unbranded and branded vegetable oil available in Khulna, Bangladesh.

MATERIALS AND METHODS

The term BVO is used to indicate edible vegetable oils which are manufactured by the registered industries whereas UVO refers to the locally produced edible oils without having a brand name. Different types of vegetable oils (7 branded and 7 unbranded), produced from a variety oilseed (black cumin oil, Sesame oil, coconut oil, sunflower oil, mustard oil, olive oil, soybean oil, and plum oil), were purchased from local markets in Khulna, Bangladesh. Acid value, Iodine value, saponification value, peroxide value, percentage of insoluble impurities and cholesterol content were determined by adopting the selected analytical methods described by American Oil Chemist's Society (AOCS) [15-17]. All chemicals (Sigma chemical company, USA) were purchased from local markets of Khulna, Bangladesh.

Acid value

1.0 g of each oil sample was weighed and poured into a conical flask containing 50 mL ethanol. Then phenolphthalein indicator (two drops) was added and titrated with 0.1 N potassium hydroxide (KOH) solution until the color of endpoint was turned into pink. The acid value was determined by using equation (1), [18].

$$Acid\ value\ = \frac{56.1 \times V \times C}{M} \qquad (1)$$

Where 56.1 is the equivalent weight of KOH, C is concentration (0.1 N) of KOH solution, V is the volume (mL) of standard KOH solution, and M is the weight of taken oil sample in gram.

Saponification value

Exact 2.0 g of each oil sample was dissolved with 25 mL ethanolic KOH in a conical flask. This solution was refluxed with continuous shaking for two hours. 1 mL phenolphthalein indicator was added and titrated with 0.5 N hydrochloric acid (HCl). For blank determination, a similar procedure was run. Saponification value was calculated by the equation (2), [18].

$$Saponification\ valu = \frac{(V_0 - V_1) \times C \times 56.1}{M} \qquad (2)$$

Where 56.1 is the equivalent weight of potassium hydroxide.V_1 is the volume of standard HCl (in mL), V_0 is the volume (mL) of standard HCl solution used for blank titration, C is the concentration of HCl and M is the mass of oil sample in gram.

Peroxide value

5.0 g of each oil sample was weighed and mixed with 30 mL of chloroform: glacial acetic acid- (2:3) in a conical flask. Then 0.5 mL of saturated potassium iodide (KI)

solution was added and allowed to stand for one minute. After that 30 mL distilled water was added and titrated with 0.01 N sodium thiosulfate ($Na_2S_2O_3$) solution using starch indicator until the yellow color was disappeared. Titration was also performed for blank determination. The equation (3) was used to calculate the peroxide value [20].

$$Peroxide\ value\ = \frac{10 \times (V_1 - V_2)}{M} \qquad (3)$$

Where, V_2 is the volume of Standard $Na_2S_2O_3$ solution used for blank titration, V_1 is the volume of standard $Na_2S_2O_3$ solution consumed by the sample oil and M is the weight of oil sample.

Insoluble Impurity

1.0 g of each oil sample was weighed and mixed with 20 mL solvent mixture of petroleum ether-diethyl ether in a ratio of 1:1 respectively in a 250 mL conical flask. This flask was shaken vigorously and allowed to stand for 30 minutes at 30 °C. Then the mixed liquid was filtered through a clean, dried and weighed Whatman 1 filter paper. The filter paper was carefully washed with the solvent mixture. After that, the filter paper was dried at 103 °C in an oven until a constant weight was obtained. The increased weight denoted impurities weight. The equation (4) was used to calculate the percentage of insoluble impurity [21].

$$\%\ of\ impurity\ = \frac{A}{W} \times 100 \qquad (4)$$

Where, A indicates the weight of filter paper and W is the weight of oil sample

Determination of Cholesterol Content

Cholesterol content in edible oil was quantified with Liebermann-Burchard reagent [22]. About 2 mg/mL of standard cholesterol solution was used as a stock solution. The reagent of Liebermann-Burchard was prepared by mixing 5mL glacial acetic acid and 7 mL concentrated sulfuric acid and kept it in dark place in ice container covered with black paper.

Preparation of sample solutions

Eight volumetric flasks were marked as P_1, P_2, P_3, P_4, P_5, P_6, P_7 and P_8. Standard cholesterol solution was added as 0.4, 0.8, 1.2, 1.6, 2.0, 2.4 and 2.8 mL in seven volumetric flasks respectively whereas, flask eight was kept blank. 2 mL of Liebermann-Burchard reagent was added in each volumetric flask and the mixture of each volumetric flask was diluted to 10 mL by adding chloroform (Table 1). All flasks were covered with black carbon paper and kept in dark for 15 min. Then, spectrophotometer with blank (P_8) was set zero at 640 nm. The absorbance was measured for all standards (eight flasks) by SP65 UV-Vis spectrophotometer and plotted the calibration curve. After that, 1 mL of oil sample, 2mL Liebermann-Burchard reagent and 7 mL chloroform were mixed for taking absorbance of each oil sample by SP65 UV-Vis Spectrophotometer. Cholesterol concentration of each sample solutions was determined using the calibration curve which was plotted by absorbance (nm) vs cholesterol concentration (mg/L).

Table 1. Preparation of Liebermann-Burchard reagent for cholesterol estimation.

Reagents (mL)	P_1	P_2	P_3	P_4	P_5	P_6	P_7	P_8
Standard cholesterol solution	0.4	0.8	1.2	1.6	2.0	2.4	2.8	-
Liebermann-Burchard reagent	2.0	2.0	2.0	2.0	2.0	2.0	2.0	2.0
Chloroform	7.6	7.2	6.8	6.4	6.0	5.6	5.2	8.0

Data on utilization of vegetable oil

Information on the utilization of the fourteen vegetable oils by the community was collected from 300 households in Khulna, Bangladesh. According to the survey data, the percentage of consumption by the people for each vegetable oil group was computed and plotted as a pie chart.

RESULT AND DISCUSSION

Presence of an elevated concentration of cholesterol in the human body leads to heart attacks, strokes and other detrimental health problems [23-26]. People consume different types of branded (BVO) and unbranded (UVO) vegetable oils directly or as their food ingredients. Different manufacturing companies claim that these BVO and UVO oils are cholesterol-free. According to the present study, it is evident that the unbranded and branded vegetable oils have higher cholesterol levels and different physicochemical properties. The experimental data for physicochemical parameters and cholesterol content of edible oil has been presented in Table 2.

Table 2. Acid value, saponification value, iodine value, peroxide value, insoluble impurity and the amount of cholesterol in fourteen oil samples.

	Sample	Acid Value (mg KOH/g)	Saponification Value (mg KOH/g)	Iodine Value (g/100g)	Peroxide Value (meq.O_2/kg)	Insoluble Impurity (%)	Cholesterol (mg/L)
BVO	Black Cumin Oil	8.56 ± 0.082	208.12 ± 0.461	88.09 ± 0.250	7.5 ± 0.103	0.11 ± 0.122	485.25 ± 0.38
	Sesame Oil	6.53 ± 0.129	188.81 ± 0.626	95.68 ± 0.313	3.91 ± 0.015	0.13 ± 0.867	329.44 ± 0.49
	Coconut Oil	4.35 ± 0.023	291.55 ± 0.968	7.56 ± 0.132	0.30 ± 0.005	0.08 ± 0.133	289.80 ± 0.99
	Mustard Oil	3.58 ± 0.026	194.63 ± 0.584	84.96 ± 0.336	0.81 ± 0.005	0.10 ± 0.384	210.07 ± 0.74
	Olive Oil	2.73 ± 0.121	195.45 ± 0.394	80.64 ± 0.474	5.93 ± 0.068	0.11 ± 0.958	165.63 ± 0.53
	Sunflower Oil	2.16 ± 0.160	181.35 ± 0.887	103.03 ± 0.39	0.50 ± 0.011	0.09 ± 0.475	145.36 ± 0.73
	Soybean Oil	1.35 ± 0.101	191.57 ± 0.447	105.73 ± 0.57	1.15 ± 0.012	0.12 ± 0.652	286.36 ± 0.24
UVO	Black Cumin Oil	9.26 ± 0.133	188.13 ± 0.124	89.04 ± 0.554	14.34 ± 0.017	0.15 ± 0.120	525.49 ± 0.67
	Sesame Oil	7.48 ± 0.132	190.71 ± 0.485	93.48 ± 0.384	4.09 ± 0.008	0.15 ± 0.311	442.79 ± 0.74
	Coconut Oil	4.90 ± 0.124	295.67 ± 0.584	8.20 ± 0.482	0.53 ± 0.008	0.12 ± 0.274	381.94 ± 0.73
	Mustard Oil	3.68 ± 0.302	180.97 ± 0.681	87.75 ± 0.530	1.19 ± 0.005	0.12 ± 0.124	470.27 ± 0.55
	Olive Oil	2.73 ± 0.056	200.93 ± 0.468	83.26 ± 0.680	6.28 ± 0.102	0.13 ± 0.539	266.39 ± 0.73
	Plum Oil	1.18 ± 0.081	208.35 ± 0.593	46.19 ± 0.584	2.00 ± 0.008	0.13 ± 0.283	282.78 ± 0.77
	Soybean Oil	1.39 ± 0.104	188.63 ± 0.659	112.44 ± 0.39	1.56 ± 0.017	0.15 ± 0.321	383.88 ± 0.73

Acid Value

The acid value is an estimation of the degree of free fatty acids present in the vegetable oil. Besides, it is an indicator of edibility of oil and suitability for the usage of the paint industry. Usually, fatty acids are found in the triglyceride form and the hydrolysis of glycerides changes the acid value. This hydrolysis may occur due to the presence of humidity in oil, elevated temperature, and microorganisms. The higher acid value gives an idea about the increasing level of free fatty acids which amplified susceptibility of oils towards rancidity and decreased oils quality [27]. For good quality of oil samples, the acceptable acid value should be below 0.6 mg KOH/g [20]. The present study reports, black cumin oil (UVO) and sesame oil (BVO) have significantly higher acid value than the rest of all oil samples whereas Coconut, Mustard, Olive, Sunflower, Soybean, and Plum oils have comparatively lower acid values (Table 2). The acid values of UVOs were comparatively higher than BVOs, and the variations were statistically significant ($p < 0.05$). This investigation supports the former study of Rajko et al and according to them, unbranded vegetable oils had higher acid value than refined oils [28]. Among these samples, unbranded black cumin oil has significantly highest (9.26 ± 0.133 mg KOH/g) acid value which shows maximum free fatty acids and has a tendency to become off-flavor that is rancidity [29]. In contrast, significantly lowest acid value (1.18 ± 0.081 mg KOH/g) was recorded for unbranded plum oil. This is might be due to the presence of a high concentration of saturated palmitic acid, which is less susceptible to oxidation than unsaturated fatty acids like linoleic acid, linolenic acid, etc. [30]. The elevated level of saturated fatty acids allows oils to be stable against rancidity and give a longer shelf-life [31]. In this study, the acid values of all vegetable oil samples have higher acid value

compared to the codex standard (CODEX-STAN210-1999) (0.6 mg KOH/g).

Saponification Value

The saponification value is inversely proportional to the chain length or average molecular weight of the fatty acids [32]. Higher the saponification value corresponds to high levels of lower fatty acids (C4-C12) [29]. The present study reveals that the coconut oil contains an elevated amount of short-chain fatty acids (< C12) due to the greater saponification value, which is comparatively higher than the codex standard permissibility limit (190-209 mg KOH/g). A high portion of saponification value in unbranded coconut oil (295.67 ± 0.584 mg KOH/g) suggests that the coconut oil could be used as a raw material for soap industries. Among the studied oil samples, the saponification value of branded black cumin (208.12 ± 0.461 mg KOH/g), sesame (188.81 ± 0.626 mg KOH/g), mustard (194.63 ± 0.584 mg KOH/g), olive (195.45 ± 0.394 mg KOH/g), soybean oils (191.57 ± 0.447 mg KOH/g) and unbranded black cumin (188.13 ± 0.124 mg KOH/g), sesame (190.71 ± 0.485 mg KOH/g), olive (200.93 ± 0.468 mg KOH/g), soybean (188.63 ± 0.659 mg KOH/g) and plum (208.35 ± 0.593 mg KOH/g) oils match with regulation of codex standard permissibility level. On the other hand, branded sunflower (181.35 ± 0.887 mg KOH/g) and unbranded mustard (180.97 ± 0.681 mg KOH/g) oils have low saponification values compared to the guideline values. It denotes, they have a comparatively high amount of long-chain fatty acids.

Iodine Value

Generally, higher iodine value represents the presence of a higher degree of unsaturation in vegetable oil or fat [33]. It also indicates the stability of oils to oxidation and allows the overall unsaturation of the fat to be determined qualitatively [20, 34]. In this study, the lowest iodine value (7.56 ± 0.132g/100g) is observed in branded coconut oil while the highest iodine value (112.44 ± 0.39) is observed in unbranded soybean oil (Table 2). The lower iodine value reveals greater oxidative storage stability and low susceptibility to oxidative rancidity. Besides, it also indicates that the oil is rich in saturated fatty acids, which ensures stability against oxidation and rancidity of foods prepared with the oil [35]. Besides, the high iodine number of unbranded soybean oil indicates the presence of high polyunsaturated fatty acids, which enhances the nutritional value of food products [36]. An increase of free fatty acid contents and decrease of total unsaturation reveal the possibility of oxidation and chemical transformation of oils during storage [37].

Peroxide value

Peroxide value is an indicator of deterioration of edible oils that measures the extent of rancidity and stability of fats and oils during storage [38]. When the double bonds of unsaturated oils and fats are oxidized, peroxides are formed as an oxidized product. The peroxide value increases with the temperature, storage duration, and contact with air of the oil samples [26]. Generally, fresh oil has less than 10 meq.O_2/kg peroxide number. Moreover, peroxide values between 20-40 meq.O_2/kg indicate rancid taste and responsible for disagreeable odor [26]. In this study, peroxide values of all oil samples are in agreement with the maximum Codex standard peroxide value for vegetable oil except unbranded black cumin oil (14.34 ± 0.017 meq.O_2/Kg). This observation suggests that unbranded black cumin oil has a high content of unsaturated fatty acids, linoleic (C18:2) and oleic acid (C18:1), which are responsible for oxidative rancidity [39]. In addition, a significantly low value is observed in branded coconut oil (0.30 ± 0.005 meq.O_2/kg) and most of the oils had an agreeable odor. The peroxide value of all UVOs is higher than that of BVOs and the variations are statistically significant ($p < 0.05$).

Insoluble Impurity

Table 2 indicates that the insoluble impurity ranged from 0.08% to 0.15% in the edible oil. According to the codex standard permissibility level (CODEX-STAN210-1999) the maximum percentage of insoluble impurities in edible oils should not exceed 0.05%. The present study shows that the mean insoluble impurities of vegetable oils exceed the Codex standard level which is a matter of concern. Presence of elevated levels of impurities in the

edible oil might cause oxidation and rancidity. Besides, it affects other physicochemical parameters and reduces the nutritional values of edible oil. The amount of insoluble impurities is reflecting the efficiency of clarification during the extraction of oil. In this study, percentages of insoluble impurities of BVOs were significantly ($p < 0.05$) lower than UVOs.

Cholesterol content

Presence of elevated levels of polyunsaturated fatty acid in oils might inhibit the activity of regulatory enzyme-like hydroxymethylglutarylcoenzyme A-reductase in cholesterol [40, 41]. Cholesterol, found in the cell membrane of all cells, plays a significant role in the production of bile acids, steroid hormones as well as vitamin D. Moreover, cholesterol has great medical importance in recent years, because of its high level in the body may develop coronary heart diseases (CHDs) [41]. In this study, the concentration of cholesterol is determined in different branded and unbranded edible oil commercially available in Bangladeshi market**.** Out of these, unbranded black cumin oil sample contained significantly ($p<0.05$) highest cholesterol which was 525.49 ± 0.67 and branded sunflower oil contained significantly ($p<0.05$) lowest cholesterol (145.36 ± 0.73 mg/L) than the others (Table 2). According to the present study it can be predicted that all unbranded vegetable oils are comprised of higher cholesterol content than branded vegetable oils.

Moreover, the obtained results from this study have been compared with other similar researches and summarized in Table 3. According to this table, it can be seen that the concentration of cholesterol is comparatively too high, while the acid value, saponification value, iodine value, and peroxide value are within the range with few exceptions.

Table 3. Comparison of acid value, saponification value, iodine value, peroxide value and the cholesterol concentration oil samples with similar studies.

Acid Value (mg KOH/g)	Saponification Value (mg KOH/g)	Iodine Value (g/100g)	Peroxide Value (meq.O_2/kg)	Cholesterol (mg/L)	Reference
2.81-11.46	106.60-246.00	9.60-52.40	0.39-5.73	--	[42]
2.47-36.47	93.27-162.69	66.37-150.37	4.36-22.25	--	[43]
1.2-4.0	182.10-193.80	95.8-124.0	1.1-10.9	--	[44]
1.31-7.39	179.04-227.94	70.00-119.67	1.03-1.53	--	[36]
0.95-25.62	180.09-258.78	60.15-127.02	0.37-6.32	--	[45]
1.91-9.76	155.68-180.92	35.85-83.75	5.60-13.80	--	[46]
4.77-12.06	34.12-260.22	--	0.60-3.80	88.80-257.10	[26]
0.3-3.54	--	--	2.16-12.0	99.6-331.0	[47]
0.17-17.46	--	26.0-124.6	--	0.38-3.99	[1]
1.35-9.26	**180.97-295.67**	**7.56-112.44**	**0.30-14.34**	**145.36-525.49**	**Present study**

Recently, a survey has been conducted among the 300 families in Khulna, Bangladesh to know the usage of different types of edible of oils. The survey data reveals that, about 18% of people are using soybean oil (BVO), 15% are using palm oil (UVO), 11% are using soybean oil (UVO), 12% are using mustard oil (UVO), 11% are using mustard oil (BVO), 8% are using sesame oil (UVO), 6% are using sesame oil (BVO), 4% are usingcoconut oil (UNO), 3% are using coconut oil (BVO), 4% are using olive oil (BVO), 2% are using olive oil (UVO), 3% are using sunflower oil (BVO), 2% are using black cumin oil (UVO) and 1% are using Black cumin oil (BVO) (Figure 1). Majority of the people are purchasing soybean oil, palm oil, and mustard oil although there is no cholesterol-free edible oil in the market. Intake of cholesterol-containing oil might cause several diseases, therefore, regular monitoring of edible oils should be carried out and the government should take necessary steps to ensure the availability of cholesterol-free vegetable oil in the market.

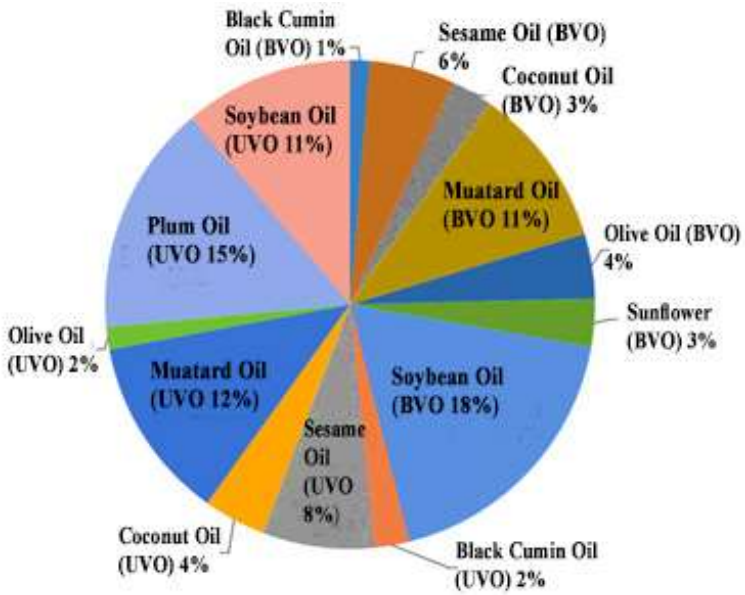

Figure 1. Percentage of people utilizing vegetable oil in Khulna, Bangladesh.

CONCLUSIONS

There are different varieties of branded and unbranded vegetable oils available in our markets and all of them claim to be cholesterol-free. But unfortunately, none of them are cholesterol-free. This study reveals that all unbranded vegetable oils are comprised of higher cholesterol content than branded vegetable oils. Due to increasing awareness on the health implications of high cholesterol in our diets, most people now prefer to purchase cholesterol-free vegetable oils. The outcomes of the present investigation express that, consuming branded sunflower oil and olive oil in our daily diet instead of other oils can achieve a remarkable degree of protection from cholesterol-oriented diseases such as heart diseases and stroke.

ACKNOWLEDGMENTS

The authors would like to thank the participants during the collection of survey data.

Conflict of interests

The authors have no conflict of interest.

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