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Physicochemical Parameters and Heavy Metal Content in Soybean Oil from Bangladesh

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Abstract: Nine familiar brands of soybean oil were selected to assess the quality of oil using standard methods of analyses. All branded oil showed satisfactory results regarding physical parameters such as moisture content, specific gravity and viscosity. However deviation from standard requirements was observed for most of the chemical parameters. Comparatively low iodine value was noticed and the values varied from 110 ± 3.0 to 114 ± 2.2 g/100 g oil. Saponification value was found in the range of 200 ± 2.9 to 210 ± 1.6 mg KOH/g which was relatively high. Five branded oil out of nine had reasonably high acid value. Peroxide value was found in acceptable range (0.85-1.42 meq/kg oil) but the value was increased rapidly by 91.2% on an average during five months storage. Cholesterol was detected in all samples and the concentration ranged from 220 ± 0.7 to 505 ± 2.1 mg/100 g oil. Lead (Pb), nickel (Ni), cadmium (Cd) and copper (Cu) contents were determined in Crude Degummed Soybean oil (CDSO) and in 9 different brands of soybean oils using atomic absorption spectrophotometer (AAS) after wet digestion. The accumulation trend for soybean oil was in the following order $Cu > Ni > Pb > Cd$. The concentration of Pb, Ni and Cu in some brands was found to be higher than the recommended legal limits. However, the estimated intakes of Pb, Ni and Cu from daily consumption of 25 g of the investigated oils should cause no risk to human health.

Key words: Acid value, cholesterol, oil oxidation, metal contamination, AAS

INTRODUCTION

All edible fats and oils are water insoluble substances that consist predominantly of glycerol esters of fatty acids, or triglycerides with a trace amount of nonglyceridic materials. The combined largest source of vegetable oils is the seeds of annual plants that grown in reasonably temperate climates (O'Brien, 2004). Vegetable fat and oil is a major source of energy providing 37 kJ/g (FAO, 2003). Oils in the diet are reachable source of fatty acids to the body which are remarkable sources of dietary calorie intake. Soybean oil is a vegetable oil extracted from the seeds of the soybean (*Glycine max*). Soybeans are grown mainly in North and South America (Brazil and Argentina). Soybean oil is one of the principal oils use for edible purposes in Bangladesh. It is imported mainly in crude form from various countries. However some quantities of soybean seed are also imported which are crushed locally to obtain soybean oil. Total import of soybean oil i.e., Crude Degummed Soybean Oil (CDSO) and crude soybean oil obtained from imported soybean together was 453,525 tonnes in 2013 (Alam, 2014). According to FAS/USDA (2015) report, Import and Domestic Consumption of soybean oil in Bangladesh was 442,000 and 530,000 tonnes, respectively in 2013/14 while world production was about 45 million tonnes for the same period. The percentage of imported CDSO and

crude soybean oil with respect to domestic consumption was above 80% during 2013/14 and the figure is consistently increasing.

Studies on fatty acid analysis of locally consumed edible oils in Bangladesh using a Gas Chromatograph (G.C) unveiled that soybean oil contained higher percentage of long chain mono and polyunsaturated fatty acids ($81.1\pm 1.5\%$) with respect to palm and coconut oil (Chowdhury *et al.*, 2007). It was also found that five fatty acids like palmitic ($C_{16:0}$), stearic ($C_{18:0}$), oleic ($C_{18:1}$), linoleic ($C_{18:2}$) and linolenic ($C_{18:3}$) acids were present in soybean oil having their composition (%) 14 ± 0.62 , 4.07 ± 0.29 , 23.3 ± 2.43 , 52.2 ± 2.64 and 5.63 ± 3.48 , respectively (Chowdhury *et al.*, 2007). Various factors such as fatty acid composition of oil, oil processing, energy of heat or light, the concentration and type of oxygen and free fatty acids, transition metals, peroxides, thermally oxidized compounds, pigments and antioxidants influence the oxidation of oil (Choe and Min, 2006). Crude soybean oil was found as most stable to the oxidation followed by deodorized, degummed, refined and bleached oil (Jung *et al.*, 1989). Crude vegetable oils have 2% or more nonglyceride substances which include phospholipids, tocopherols, sterols, resin, carbohydrates, proteins, pesticides, trace metals and pigments. Among them few nonglyceride constituents are detrimental which are subjected to

remove during refining process with least possible damage of desirable ingredients (O'Brien, 2004).

Cholesterol is the major sterol found in animals but only in trace amount in vegetable oils (Lehninger *et al.*, 2005). It has essential structural roles in membranes and lipid metabolism. In addition it plays a significant role to the development and working of the central nervous system (Christie, 2014). Two types of lipoproteins bring cholesterol to and from cells that are low-density lipoprotein (LDL) and high-density lipoprotein (HDL). Literature showed that HDL cholesterol is good cholesterol because it assists removing LDL cholesterol from the arteries while LDL contributes to plaque, a thick, hard deposit that can block arteries and make them less flexible. Phytosterols having similarity with cholesterol structure inhibit cholesterol absorption in intestine, As a result total plasma cholesterol and low-density lipoprotein (LDL) levels are decreased (Wong, 2001; Plat and Mensink, 2001). The risk for coronary heart diseases (CHD) reduces dramatically with the decline of serum LDL cholesterol level. Foods containing low saturated fat and cholesterol and high in plant sterols can decrease LDL by 20% (Katan *et al.*, 2003). The concentration of sterols and cholesterol in soybean oil was found 2965±1125 and 28±7 ppm, respectively (O'Brien, 2004). It is essential to ascertain the quantity of cholesterol present in soybean oil.

Edible oil quality concerning its freshness, storability and toxicity can be assessed by the determination of a number of trace metals (Zhu *et al.*, 2011). Quantity of trace metals like Cu and Ni are recognized to boost the rate of oil oxidation whereas other elements such as Pb and Cd are very significant on account of their toxicity and metabolic role (Anthemidis *et al.*, 2005). A number of factors such as soil, environment, genotype of the plant, fertilizers and/or metal-containing pesticides, introduced during the production process or by contamination from the metal processing equipment are responsible for the presence of metal in vegetable oils (Zeiner *et al.*, 2005; Jamali *et al.*, 2008). As per our knowledge, there has been no report on the heavy metal levels in edible soybean oils in Bangladesh. Therefore one of the main objectives of this study was to determine the concentrations of Pb, Ni, Cd and Cu in soybean oil. Moreover it is also important to assess the physical and chemical parameters in order to ensure the quality of oil consumed in Bangladesh. These physicochemical parameters include moisture content, specific gravity, viscosity, iodine value, saponification value, acid value, peroxide value and cholesterol concentration. The aim of this research was to provide information on refined soybean oil quality, to compare oil quality with the standard specifications, to create awareness among the manufacturers as well as to provide suggestion to the monitoring authority.

MATERIALS AND METHODS

We selected nine brands of soybean oil in relation to reputation of products, availability and high rate of consumption all over the country. For each brand, we collected three samples from three different location of Chittagong metropolitan city, Bangladesh in order to get clear concept about each product. All nine branded samples were coded as RS 01, SAS 02, FS 03, DS 04, TS 05, MoS 06, MuS 07, NS 08 and PS 09, respectively. Crude Degummed Soybean oil (CDSO) was collected which is imported regularly through Chittagong port. The collected oil samples were kept in plastic bottle and stored below 20°C until analysis. All chemicals and reagents used in this study were of analytical grade (supplied by Merck, Germany). Standard methods were applied in all the determinations.

IUPAC method: The IUPAC method (1979) was used in determination of moisture, specific gravity, viscosity, iodine value, saponification value, acid value, peroxide value and free fatty acid.

Cholesterol analysis: Liebermann-Burchard method (Kenny, 1952) was used to determine Cholesterol. The Liebermann-Burchard reaction method is a colorimetric method in which cholesterol is treated by means of chloroform, acetic anhydride and concentrated sulfuric acid to produce a green colour which was measured spectrophotometrically by using CINTRA double beam UV-Visible spectrometer (Serial no. V 3690, GBC Scientific Equipment Pty Ltd., Australia).

Heavy metal analysis: Analysis of Pb, Ni, Cd and Cu were carried out for CDSO and all nine branded soybean oil samples by using atomic absorption spectrophotometer (AAS) after wet digestion.

Digestion procedure: Wet digestion process was performed for the mineralization of trace heavy metals in oil samples. A three-neck, 500 mL round-bottom flask (Quickfit) with a reflux condenser was used for wet digestion. The digestion was performed for the complete destruction of the organic matrix using a mixture of HNO₃ (65%) and HClO₄ (70%) (14:2, v/v) (16 mL for a 1 g sample). The mixture was heated at 130-140°C in a heating mantle (model EM0500/CE, Electrothermal, UK) for 6 hours and then digested again with additional 10 mL 10% HNO₃ for 3 h at 175-180°C. The final volume of the solution was reduced to about 3-5 mL after complete digestion. 5 mL deionized water was added to the residue and mixed. Then the solution was filtered through a Whatman No. 40 filter paper and transferred to 50 mL volumetric flask and made up to the mark. A blank digest was carried out in the same way.

Instrumentation: Atomic absorption spectrophotometer (AAS) (Type: iCE 3300 AA system, Thermo Scientific,

designed in UK) was used to determine lead, nickel, cadmium and copper in oil sample. The analysis was carried out using respective hollow cathode lamps under standard instrumental conditions (Table 1).

RESULTS AND DISCUSSION

The physical properties of various brand samples of soybean oil were investigated and results are shown in Table 2. In presence of moisture oil can be hydrolyzed or can form rancid, as a result oil loses the taste and develops unpleasant smell. The moisture content of oil sample was within range of 0.24 to 0.46%. The amount of moisture increases gradually if the extraction of oils from seeds is not accurate and preservation process is uncontrolled. Controlled refining is very important for ensuring good quality of edible oils. The recommended moisture content in soybean oil is around 0.2-0.3% (Hui, 1996; Gupta, 2008; Daun *et al.*, 2011) which is comparable with the moisture content found in the tested samples.

The specific gravity (SG) of all fats and oils virtually observe between 0.90-0.95 (Islam *et al.*, 2008). The SG value obtained for all sample was varied from 0.91 to 0.92 at 30°C. The Specific gravity results indicate the purity of the oil samples and it can be said that probably most of the oil samples are well refined. Fats or oils possess relatively high viscosity due to the intermolecular attractions of the long chains of the glycerides molecules. Viscosity increases with molecular weight but decreases with increasing unsaturated level and temperature (Noureddini *et al.*, 1992). Viscosity (at 30°C) of oils was found from 29.6 cP in NS 08 to 39.9 cP in SAS 02 sample. In general the results give an idea about the intermolecular hydrogen bonding in oil (Kirk and Othmer, 2008). Viscosities had been measured for a number of vegetable oils as a function of temperature by Noureddini *et al.* (1992) and the results for soybean oil were 54.3, 31.8 and 23.3 cP at temperature 23.9, 37.8 and 48.9°C, respectively.

Industrial parameters such as iodine value, saponification and acid value of oil indicate the oxidative stability, functionality, saturation, unsaturation and fatty acid composition. The results are shown in Table 3. Iodine value (IV) is a simple chemical constant for a fat or oil (Shahidi, 2005). It measures the average number of double bonds in fats and oils. The oxidative and chemical changes in oils during storage are characterized by an increase in free fatty acid contents and a decrease in the total unsaturation of oils (Perkins, 1992).

Soybean oil has comparatively high iodine value which means more double-bonds. The range of IV value for most of the commercial soybean oil is from 124 to 136 while the range for palm oil is from 48 to 60 (Jamieson, 1943). IV value in all tested sample ranged in between 110±3.0 and 114±2.2 g/100 g oil. These values indicate

Table 1: Operating conditions of AAS

Flame type	Air-Acetylene
Number of resamples	3
Measurement time (sec)	4
Burner height (mm)	7
Fuel flow (L/min)	1.1 (Pb and Cu), 0.9 (Ni), 1.2 (Cd)
Bandpass (nm)	0.5 (Pb, Cd and Cu), 0.2 (Ni)
Lamp current (%)	75 (Pb, Ni and Cu), 50 (Cd)
Elements monitored (wavelength, nm)	Pb (217), Ni (232), Cd (228.8), Cu (324.8)
Instrument detection limits (ppm)	0.013 (Pb), 0.008 (Ni), 0.0028 (Cd), 0.0045 (Cu)

Table 2: Moisture content, specific gravity and viscosity of tested soybean oil samples

Sample code (n = 3)	Moisture content (%)	Specific gravity at 30°C	Viscosity (cP) at 30°C
RS 01	0.24±0.02	0.91±0.01	33.7±0.06
SAS 02	0.33±0.03	0.91±0.01	39.9±0.01
FS 03	0.36±0.02	0.91±0.01	37.6±0.01
DS 04	0.45±0.02	0.92±0.01	39.7±0.03
TS 05	0.27±0.02	0.91±0.01	37.4±0.08
MoS 06	0.27±0.01	0.92±0.01	38.3±0.10
MuS 07	0.34±0.03	0.91±0.01	36.1±0.07
NS 08	0.31±0.02	0.92±0.01	29.6±0.26
PS 09	0.46±0.02	0.91±0.01	35.4±0.32

n: Number of observations; Mean values±standard deviation

Table 3: Iodine value, saponification value and acid value of tested soybean oil samples

Sample code (n = 3)	Iodine value (g/100 g oil)	Saponification value (mg KOH/g)	Acid value (mg KOH/g)
RS 01	114±2.2	202±3.3	0.63±0.13
SAS 02	112±2.0	210±1.6	0.50±0.07
FS 03	112±1.5	205±2.2	0.39±0.05
DS 04	111±1.3	208±1.8	0.70±0.17
TS 05	112±1.4	203±1.4	0.49±0.06
MoS 06	111±1.0	205±1.2	0.70±0.27
MuS 07	110±3.0	208±1.7	0.64±0.29
NS 08	111±2.7	200±2.9	0.29±0.08
PS 09	111±1.2	206±1.8	0.82±0.12

n: Number of observations; Mean values±standard deviation

Table 4: Peroxide value and cholesterol concentration of tested soybean oil samples

Sample code (n = 3)	Peroxide value (meq/kg oil)	Cholesterol concentration (mg/100 g oil)
RS 01	1.19±0.13	337±0.5
SAS 02	1.27±0.07	401±0.9
FS 03	1.10±0.10	441±1.6
DS 04	1.10±0.04	360±1.7
TS 05	0.85±0.02	285±2.6
MoS 06	1.42±0.04	361±2.0
MuS 07	1.21±0.13	237±1.1
NS 08	1.13±0.03	220±0.7
PS 09	1.05±0.14	505±2.1

n: Number of observations; Mean values±standard deviation

that all samples contained moderate proportion of unsaturated fatty acids and were of semi drying type. According to Bangladesh Standard (1979), IV value requirement for soybean oil is 120-143. However the mean value was lower than that of standard range which suggests that the oil samples may be blended with other less unsaturated and economically cheap

vegetable oil (e.g., palm oil). Saponification value (SV) is an index of average molecular mass of fatty acid in oil (Zahir *et al.*, 2014). It is inversely proportional to the average molecular weight or chain length of fatty acids present in oil. The range of standard SV value of refined soybean oil is 189-195 mg KOH/g (Bangladesh Standard, 1979). Highest and lowest SV value was found 210 ± 1.6 in SAS 02 and 200 ± 2.9 in NS 08 sample, respectively. High saponification value indicates that oil was not pure and well refined. Acid value (AV) is an important property of oil to indicate the quality, age, edibility and suitability of oil for uses. It is used to measure the extent to which glycerides in the oil has been decomposed by lipase and other physical factors such as light and heat (Hilditch, 1949). Thus, the higher AV value suggests that the oil sample is more susceptible to lipase action (Deman, 1990). The lowest and highest AV value was found in NS 08 (0.29 mg KOH/g) and PS 09 (0.82 mg KOH/g) sample, respectively. According to standards, maximum level of AV value in refined oil is 0.6 mg KOH/g oil (Bangladesh Standard, 1979; Codex Alimentarius Commission, 2001) but in our study the AV value was found higher than the maximum limit in five (RS 01, DS 04, MoS 06, MuS 07, PS 09) sample out of nine branded sample. Low acid value is an indication of freshness and suitability of oil or fat for edible purpose while high acid value indicate the rancidity which is caused by hydrolysis of the ester links and oxidation of double bonds of the triglycerides.

The biochemical parameters such as peroxide value and cholesterol concentration were studied which are presented in Table 4. Peroxide value (PV) is the most common measurement of lipid oxidation. Hydroperoxides have no flavor or odor of their own, but they are unstable and break down rapidly to other products such as aldehydes that have a strong, disagreeable flavor and odor (Shahidi, 2005). It is used to assess in what extent rancidity reactions have been occurred during storage, it could be used as an indication of the quality and stability of fats and oils (Ekwu and Nwagu, 2004). The highest PV values were found in MoS 06 (1.42 ± 0.04 meq/kg oil) sample while the lowest were observed in TS 05 (0.85 ± 0.02 meq/kg oil). The values for all oil samples were within the Codex Alimentarius Commission (2001) standards. Peroxide values increased during storage. In general the PV value increased with storage time. Oils exposed to both atmospheric oxygen and light showed a dramatic increase in peroxide value during storage. Change of peroxide value with storage time had also been studied which are shown in Fig. 1. The average increased of PV value of soybean oil was 91.2% after five months. The raise of PV value was over 100% for FS 03, DS 04 and TS 05 within five months duration. Auto oxidation reactions may take place rapidly in oils that contain

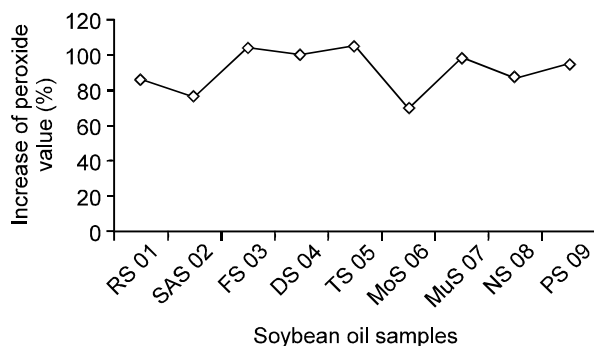


Fig. 1: Change of Peroxide value of soybean oil samples after five months storage

mainly unsaturated fat molecules and the reactions occur at an increasing rate after the initial induction period (Aluyor and Ori-Jesu, 2008). The oxidation of oil could be prevented with the inclusion of a natural or synthetic antioxidant (Bera *et al.*, 2006). Synthetic antioxidants such as Tertiary butyl hydroquinone (TBHQ), Butylated hydroxyanisole (BHA) or Butylated hydroxytoluene (BHT) recommended by FAO/WHO may be introduced in order to enhance the storage properties of oil (Alimentarius, 1999). Oils samples stored in airtight condition in the dark had comparatively lower increase in peroxide value compared to oils samples exposed to both atmospheric oxygen and light. Cholesterol in food increases the level of LDLs (Johnson and Greenland, 1990). We found cholesterol in all tested soybean oil sample. The high cholesterol content was detected in PS 09 sample (505 ± 2.1 mg/100 g oil) while the lowest amount was identified in NS 08 (220 ± 0.7 mg/100 g oil). Sample of five brands had cholesterol levels higher than 350 mg/100 g oil while cholesterol levels in remaining four brand samples ranged between 220 and 337 mg/100 g oil. Cholesterol was found in rapeseed oil samples in Finland and in edible fats and oils samples in Pakistan (Toivo *et al.*, 1998; Sabir *et al.*, 2003). An analysis of different brands of vegetable oils samples in Nigeria showed that cholesterol was present in most of the oil brands samples in the range of 0.39-4.0 mg/mL (Okpuzor *et al.*, 2009).

The presence of heavy metals in soybean oil was studied and the results are shown in Table 5. Firstly the accuracy of the wet digestion procedure was checked through spike recovery test. Detection limit values of elements as ppm were found to be 0.12 for Pb, 0.16 for Ni, 0.11 for Cd and 0.019 for Cu in this study. The spike recovery values were almost suitable ($\geq 97\%$) for wet digestion method and the relative standard deviations were less than 5% for all examined elements. Maximum dietary intake of a single type of fat or oil is 25 g for an adult (Zhu *et al.*, 2011). Lead (Pb) has no favorable role in human metabolism and creates progressive toxicity

Table 5: Amounts ($\mu\text{g/g}$) of Pb, Ni, Cd and Cu in soybean oil

Oil type	Sample code	Metal content ($\mu\text{g/g}$) \pm SD			
		Pb	Ni	Cd	Cu
Crude degummed soybean	CDSO	BDL	0.32 \pm 0.02	BDL	9.75 \pm 0.11
Refined soybean	RS 01	2.35 \pm 0.06	18.5 \pm 0.12	BDL	30.5 \pm 0.16
	SAS 02	0.31 \pm 0.02	8.5 \pm 0.07	BDL	14.5 \pm 0.09
	FS 03	BDL	10 \pm 0.05	BDL	12 \pm 0.11
	DS 04	0.45 \pm 0.01	11 \pm 0.03	BDL	13 \pm 0.14
	TS 05	0.55 \pm 0.04	8 \pm 0.08	BDL	18 \pm 0.08
	MoS 06	1.3 \pm 0.02	11 \pm 0.05	BDL	16 \pm 0.06
	MuS 07	BDL	7.7 \pm 0.1	BDL	19 \pm 0.12
	NS 08	BDL	8.7 \pm 0.09	BDL	18 \pm 0.08
	PS 09	0.95 \pm 0.01	6.8 \pm 0.04	BDL	22 \pm 0.14

BDL indicates below detection limit, SD indicates standard deviation of three replicate measurements

(Zhu *et al.*, 2011). Pb was not found at the ppm level in crude degummed soybean oil (CDSO) but it was present in six branded refined oil out of nine. Pb contents were in the range of 0.31-2.35 $\mu\text{g/g}$ which were higher than those in previous reports (Dugo *et al.*, 2004; Zhu *et al.*, 2011). According to international requirements, the approved contents of Pb, Ni, Cd and Cu in oils are 0.1, 0.2, 0.05 and 0.1 $\mu\text{g/g}$, respectively (FAO/WHO, 2011; Kowalewska *et al.*, 2005). For an average adult (60 kg body weight), the provisional tolerable daily intake (PTDI) for Pb is 214 $\mu\text{g/g}$ (FAO/WHO, 1999). Nickel (Ni) at trace level may be effective as an activator of some enzyme systems however its toxicity at higher concentrations is more well-known (Zhu *et al.*, 2011). Ni contents were found in the soybean oil samples in the range of 0.32-18.5 $\mu\text{g/g}$ which was higher than those found in the literature for Turkish edible oils (Pehlivan *et al.*, 2008). WHO recommends 100-300 $\mu\text{g/g}$ nickel for daily intake (World Health Organization, 1994). Cadmium (Cd) is a very toxic metal and excessive cadmium exposure may arise various health disorders such as renal, pulmonary, hepatic, skeletal, reproductive effects and cancer (Zhu *et al.*, 2011). Cd was not detected at the ppm level in any of the analyzed samples though Cd was present in Turkish edible oils (Pehlivan *et al.*, 2008) and Chinese vegetable oils (Zhu *et al.*, 2011). Copper (Cu) is vital for maintaining good health however very high intake can be reason for undesirable health problems such as liver and kidney damage (Ikem and Egiebor, 2005). In addition rancidity of oil may accelerate with the presence of excess Cu which act as a catalyst (Jamieson, 1943). The Cu content of the samples were ranged from 9.75 to 30.5 $\mu\text{g/g}$. In the literature Cu levels in edible oil samples were described in the range of 0.02-0.33 $\mu\text{g/g}$ (Garrido *et al.*, 1994; Zhu *et al.*, 2011), 12.7-50.5 (Buldini *et al.*, 1997; Ajayi *et al.*, 2006). The PTDI for Cu is 3 mg/g for an average adult (60 kg body weight) (FAO/WHO, 1999).

Conclusion: With respect to physical parameter, all tested soybean oil samples seemed to be well refined. However low iodine value, high saponification value and

acid value of most of the tested oil suggest that the oil were not well refined and producers may have blended soybean oil products with other cheap oil products. Sharp change of peroxide value during storage may happen due to the presence of unsaturated fat molecules and transition metal ions in oil and removal of antioxidant during refining of oil. Antioxidant may be introduced in manufactured soybean oil in order to prevent the oxidation of oil during storage. Our study reveals noticeable amount of cholesterol in all soybean oil samples, though edible oil manufacturers advertise their products as 'Cholesterol Free'. First time we analyzed heavy metal in refined soybean oils in Bangladesh and detected harmful metals such as Pb and Ni at high concentration. Treatment processes regarding refining and packaging procedures may be responsible for heavy metal contamination in oil. Oil producer should take further care in refining process in order to improve the quality of oil following the standard specification. In addition people should consume oil considering the quality of oil.

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