Chemometric identification of a few heavy metals, pesticides and plasticides in edible sunflower oil for health risk assessment

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ABSTRACT
Sunflower oil demand is increasing, pertaining to its vital qualities. The quality of sunflower oil cannot be ascertained with adulteration detection only. It also includes the detection of the presence of other harmful substances, such as pesticides, plasticides, and heavy metals; which are commonly known as Endocrine Disrupting Chemicals [EDC]. The above chemicals even at a trace level are harmful, for consumption. The proposed work was to detect the presence of EDC from sunflower oil. The results after analysis revealed the contamination of heavy metals ranging from mercury (0–82.2 ppm), arsenic (0–0.97 ppm), cadmium (0–0.48 ppm), and lead (below detectable limit). QuEChERS method was used to detect the presence of pesticides (Permethrin, N, N-Dimethyldecanamide) and plasticides (Bisphenol, Diocetyl Phthalate-DIOP, Dibutyl phthalate) from sunflower oil. The work also identified the health risk on regular consumption of sunflower oil containing the above compounds.

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Introduction

Sunflower oil is recommended for diabetic and cardiac patients [1,2] as it contains more of polyunsaturated fatty acid that lowers bad low density level cholesterol and has 66% of linoleic acid that washes away cholesterol from the coronary artery. India has witnessed the epidemic dropsy due to argemone oil adulteration with mustard oil that led to various measures enforced by Bureau of Indian Standards [BIS] to market edible oils in packaged forms using plastic pouches, plastic cans. These measures can prevent edible oil adulteration with other inedible oils. As no standard procedures are available to check the presence of Endocrine Disrupting Chemicals [EDC] in food[3,4], regular consumption of the sunflower oil adulterated with EDC can lead to various disorders[5] like obesity, precocious puberty, and cancer. The serious threat such as diabetes, obesity that our society faces in the present day, could be due to the consumption of food with EDC.

Edible oil is consumed every day for cooking and frying. Import of sunflower oil in India, has increased to 35% in the recent oil years. As edible oils are marketed in packeted form, the plasticidal materials such as Bisphenol and its derivatives fuse into the oils. Industrial wastes that are drained into the soils can lead to the accumulation of heavy metals in the soil. Machiner is used for oil manufacturing. The oil seeds come in contact with the various heavy metals lead, cadmium, mercury in the machines.[6,9] The excessive use of pesticides[10,11] to the crops and during storage, result in residues that remain even in oils.

The pesticides[12–15], plasticides and heavy metals[16] cannot be detected from edible oil with simple analysis but require more sophisticated instruments such as Gas Chromatography/Mass
Spectrometry [GC/MS] and Atomic Absorption Spectroscopy [AAS]. Chemometrics [17] with multivariate [18] techniques can be employed to detect EDC in edible sunflower oils. The aim of the present work is to combine the results from GC/MS and AAS for identifying health risk index [19,20] on consuming sunflower oil regularly with various heavy metals such as mercury, arsenic, cadmium, and lead along with other pesticides and plasticides.

**Materials and methods**

Sunflower oil packaged in pouches and plastic cans of different brands were purchased from markets of Tamil Nadu, India for analysis. Eleven brands of sunflower oil were selected from different locations. Among them 90 samples from the 11 brands were tested. Each sample was analysed thrice for accuracy.

**Sample preparation for GC/MS analysis**

Pesticides and plasticides cannot be detected with usual analysis as they are in trace levels hence require QuEChERS—(Quick, Easy, Cheap, Effective, Rugged, and Safe) method [21] for extraction. GC/MS with QuEChERS kit (Agilent dispersive kit part no: 5982–0028) was used for extracting pesticide and plasticide residues from sunflower oil samples. The steps employed for extraction is given as follows. Take 3 g sunflower oil in a test tube. Add 7 ml water, 10 ml Acetonitrile, 4 g of anhydrous Magnesium sulphate, and 1 g of Sodium Chloride to it. Centrifuge the tube at 3450 rpm for 1 min. Transfer 1 ml of the extract to the Agilent dispersive kit and shake well. Again Centrifuge at 3450 rpm for 1 min. The supernatant is taken for GC/MS analysis.

GC/MS (PerkinElmer Clarus 500) instrument was used for analysis and the conditions are as follows: Software: Turbomass ver5.2.0, Column Type: Capillary Column Elite-5MS (5%Phenyl 95% dimethylpolysiloxane), Column length, inner diameter: 30m, 250μm GC Conditions: Oven Program: 50 °C@6°C/min to 200 °C(2 min)@6 °C/min to 270 °C(10 min), Injector temp: 280 °C, Carrier gas: Helium @ flow rate 1ml/min, Split ratio: 1:10. MS Conditions: Mass Range: 40–600 amu, Type of ionisation: Electron Ionisation, Electron energy: 70ev, Transfer line and source temperature: 200°C, 180°C. QuEChERS extracts the pesticide, plasticide residues from the samples are subjected to GC/MS analysis. The compounds elute at different times based on weight and affinity to the column. Quechers contains dispersive primary secondary amine is used to achieve good recoveries from the target samples. The recovery ranged from 80 to 90% with standard deviation ranging from (0.01–2) for all the samples indicating acceptable precision of the methods used. A sample chromatogram of sunflower oil sample that contains plasticide and pesticide residue is given in Fig. 1. The detected EDC

![Figure 1. Chromatogram of Sunflower oil showing various residues.](image-url)
compounds with IUPAC name, common name, formula, molecular weight, and elution time are given in Table 1.

Heavy metal detection

The heavy metal detection is performed for the oil samples using AAS. The liquid samples are reduced to ash using Muffle furnace at a temperature range of 450°C–500°C for 4–5 h. The ash is digested with concentrated nitric acid and distilled water in ratio 1:1 on a hot plate for 4–5 h. After cooling it was made into 100ml, filtered before AAS analysis. The results obtained for one sample is given below in Table 2. Figure 2 graph shows the presence of various EDC in the sunflower oil.

Results and discussion

In Table 3, the results of contamination (mean ± SD), Maximum Allowable Limit for EDC compounds in ppm according to BIS regulations, heavy metal (Hg, Cd, Ar, and Pb) residues for the same samples identified using AAS are given. Sunflower oil brands are indicated as B with a number (B1, B2…B11), BDL indicates Below Detectable limit.

Table 1. EDC compounds detected using GC/MS for sunflower oil.

| S. No | IUPAC Name | Common Name & Formula | Molecular Weight | Elution Time |
|-------|------------|------------------------|------------------|--------------|
| 1     | 3-Phenoxybenzyl (1RS)-cis,trans-3-(2,2-dichlorovinyl)-2,2-di methyl cyclo propane carboxylate | Permethrin C21H20Cl2O3 | 390 | 23.87 |
| 2     | N, N-Dimethyl decanamide | No Common name C12H25NO | 199 | 33.03 |
| 3     | Bis(2-ethylhexyl) phthalate (di-2-ethylhexyl phthalate) | Di octyl Phthalate DOP C24H36O4 | 390 | 37.05 |
| 4     | o-Benzene dicarboxylic acid dibutyl ester | Dibutyl Phthalate (DBP) C16H22O4 | 278 | 37.12 |
| 5     | 4,4’-(p-Phenylene)di isopropylidene)diphenol | Bisphenol P C24H26O2 | 346 | 39.43 |

Table 2. Heavy metals detected from sunflower oil Using AAS.

| S.no | Heavy Metal | Symbol | Detected (ppm) |
|------|-------------|--------|----------------|
| 1    | Mercury     | Hg     | 80             |
| 2    | Cadmium     | Cd     | 0.48           |
| 3    | Arsenic     | As     | 0.97           |
| 4    | Lead        | Pb     | Below Detectable Limit |

Figure 2. Graph showing various EDC compounds in sunflower oil.
Reasons for heavy metals, pesticides and plasticides in sunflower oil

In India, permethrin is used for storage of sunflower oil seeds. As a result permethrin remains as a residue in the oil. Sunflower is grown as an intercrop and the various pesticides sprayed to the main crop which attributes to their presence in the sunflower oil. The WHO and FAO recommend 1 ppm residue limit for pesticides in sunflower oil. During packaging into pouches and cans various phthalates, Bisphenol P are the compounds elute into the oil from it. In Salem, Erode districts of Tamil Nadu, India sunflower crop is grown around handloom and textile units. Heavy metals such as mercury, cadmium in the oil could be due to the cultivation of sunflower crop near industrial wastelands.

Health risk associated with consumption of sunflower oil

Huge doses of permethrin a neurotoxin \(^{24}\), affects the neuron membranes. Carbofuran is a highly toxic and an endocrine disruptor. \(^{25}\) It causes alterations in hormones. At 0.4 ppm observations show a decline in sperm count and motility for men. Phthalates \(^{26}\) are very harmful to children that are abundantly found in fatty foods. They cause obesity, early menarche, metabolic interference, and hormonal disruption. DIOP is an organophosphorus pesticide harmful to children. \(^{27}\) Due to organophosphorus pesticide contamination in the free mid-day meal in Bihar, India, 23 school children died. Bisphenol P is a plastic derivative and a xenoestrogen low dose exposure cause hormonal disruption and lead to breast cancer for women. \(^{28}\)

The mercury toxicity \(^{29}\) varies depending on the dosage and exposure. The symptoms are sensory impairment and lack of coordination of movements. This may lead to the damage to liver, brain, lungs, kidneys, and several other diseases like pink disease, Minamata diseases etc. Cadmium is resistive to corrosion and it is one of the six hazardous substance banned in Europe with a few exemptions. Cadmium accumulation may lead to deficiency of iron and other minerals, itai-itai \(^{30}\) disease, lung and prostate cancer. Arsenic is toxic heavy metals and affects bones and kidneys, causes peripheral vascular diseases, Taiwan’s blackfoot diseases, hypertension, cancer, cardiovascular \(^{31}\) diseases etc., Lead is another poisonous heavy metal harmful for children. For adults it causes insomnia\(^{32}\), nerve damage and various other problems.

Health risk modeling

Indian edible oil consumption is around 39 g per day whereas only 29 g per day is suggested. Sunflower oil consumed at this rate regularly accounts to consuming 1170 g per month. The

### Table 3. EDC contamination in sunflower oil.

| Samples | Permethrin | NN | DIOP | Pthalates | Bisphenol | Hg | Cd | Ar | Pb |
|---------|------------|----|------|-----------|-----------|----|----|----|----|
| MAL (ppm) | 1 | 5000 | 1.5 | 1.5 | 3 | 1 | 15 | 0.12 | 0.5 |
| B1 | 1.36 ± 0.03 | 0.49 ± 0.01 | 45 ± 2 | 33 ± 1 | 11.3 ± 0.2 | 81.4 ± 1 | 0.48 ± 0.01 | BDL | 0 |
| B2 | 1.26 ± 0.03 | BDL | BDL | 12 ± 0.01 | 5.6 ± 0.3 | 76.3 ± 1 | 0.23 ± 0.1 | 0.97 ± 0.01 | BDL |
| B3 | BDL | BDL | BDL | 13 ± 0.02 | 4.0 ± 0.3 | BDL | BDL | BDL | BDL |
| B4 | 1.01 ± 0.03 | BDL | BDL | 13.3 ± 0.01 | 1.2 ± 0.2 | 82.2 ± 1 | 0.54 ± 0.1 | BDL | BDL |
| B5 | BDL | BDL | BDL | 0.92 ± 0.01 | 0.9 ± 0.3 | BDL | BDL | 0.24 ± BDL | BDL |
| B6 | BDL | BDL | BDL | 0.3 ± 0.1 | BDL | BDL | BDL | BDL | BDL |
| B7 | 0.08 ± 0.04 | BDL | BDL | BDL | BDL | BDL | 0.52 ± 0.01 | BDL | BDL |
| B8 | BDL | BDL | BDL | 1.1 ± 0.2 | BDL | BDL | BDL | BDL | BDL |
| B9 | BDL | BDL | BDL | BDL | BDL | BDL | BDL | BDL | BDL |
| B10 | BDL | BDL | BDL | 1.7 ± 0.01 | BDL | BDL | BDL | BDL | BDL |
| B11 | BDL | BDL | BDL | BDL | BDL | BDL | BDL | BDL | BDL |

MAL: Maximum Allowable Limit  BDL – Below Detectable Limit
projected consumption of an individual for the same is given in Table 4. Health Risk index is calculated based on edible oil consumption for an average Indian adult (60 kg) using the Eqs. (1) and (2) as suggested by WHO is given below:

\[
\text{Health Risk Index} = \frac{\text{Daily Estimated Oil Intake}}{\text{Acceptable Daily Oil Intake}}
\]

\[
\text{Daily Estimated Oil Intake} = \frac{\text{Residual EDC concentration (ppm)} \times \text{Oil consumption rate (g/day)}}{\text{Average Body weight (60kg)}}
\]

The percentage of contamination of various EDC in the investigated sunflower oils along with the projected consumption of EDC compounds (maximum residues) for 1 month (daily intake for 30 days) is shown in Table 5.

**Conclusion**

The presence of DIOP, phthalates and mercury pose a greater threat for health, as the health risk index associated is greater. Similarly, residues of other EDC compounds are in greater level when taken on regular basis. Mercury levels pose a greater health risk and also the presence of other compounds even in low level ppm act as toxins in our body. Hence government should impose norms to control and check the presence of these compounds. Sunflower should not be cultivated near industrial areas to prevent heavy metals entering into the roots and into the seed. Application of permethrin usage should be checked on storing the seed. The packaging materials should be a food grade material. For common man, it is also recommended to change the type and brand of oil every month to avoid chronic exposure to the EDC.

**Table 4.** Projected Consumption of EDC compounds in Sunflower oil.

| Compound      | Presence mg/kg | 39 gms consumption per day |
|---------------|----------------|----------------------------|
|               | one month      | four month                 | Nine months | One year |
| Permethrin    | 1.36           | 1.59                       | 6.36        | 14.32    | 19.09    |
| NN dimethyl decanamide | 0.49           | 0.57                       | 2.29        | 5.16     | 6.88     |
| DIOP          | 45             | 52.65                      | 210.60      | 473.85   | 631.80   |
| Phthalates    | 33             | 38.61                      | 154.44      | 347.49   | 463.32   |
| Bisphenol P   | 11.3           | 13.22                      | 52.88       | 118.99   | 158.65   |
| Mercury       | 81.4           | 95.24                      | 380.95      | 857.14   | 1142.8   |
| Cadmium       | 0.48           | 0.56                       | 2.25        | 5.05     | 6.74     |
| Arsenic       | 0.97           | 1.13                       | 4.54        | 10.21    | 13.62    |
| Lead          | 0              | 0                          | 0           | 0        | 0        |

**Table 5.** Health Risk assessment on Sunflower oil with EDC compounds.

| Residues      | Permethrin | NN | DIOP | Phthalates | Bisphenol P | Hg | Cd | Ar | Pb |
|---------------|------------|----|------|------------|--------------|----|----|----|----|
| Contamination rate % | 36 | 9 | 27 | 54 | 45 | 27 | 36 | 18 | 0 |
| Projected Consumption for One month (39 gms of oil/day) | 1.59 | 0.57 | 52.65 | 38.61 | 13.22 | 95.24 | 0.56 | 1.13 | 0 |
| Health Risk Index for One Month | 0.053 | 0.00003 | 1.17 | 0.86 | 0.15 | 3.17 | 0.001 | 0.32 | 0 |
| Health Risk | No | No | Yes | Yes | No | Yes | No | No | No |
References

[1] Mishra, S.; Manchanda, S. C. Cooking Oils for Heart Health. Journal of Preventive Cardiology 2012, 1, 3, 123–131.

[2] Radha, M. B.; Kanaka Durga Devi, N.; Sai Mrudula, B.; Nagendra, B. R. The Importance of Biodegradable Bio-Oil – SUNFLOWER. International Journal of PharmTechResearch 2010, 2, 3, 1913–1915.

[3] Fritsche; Hans, S.; Steinhart. Occurrence of Hormonally Active Compounds in Food: A Review. A Review, European Food Research and Technology 1999, 209, 153–179. DOI: 10.1007/s002170050475.

[4] O’Connor John, C.; Robert, E. C. Critical Evaluation of Observed Reproductive Effects of Endocrine Active Substances on Reproduction and Development, the Immune System, and the Nervous System. Pure And Applied Chemistry 2003, 75, 2099–2123. DOI: 10.1351/pac200375112099.

[5] Balabanic, D.; Rupnik, M.; Klemencić, A. K. Negative Impact of Endocrine-Disrupting Compounds on Human Reproductive Health. Reproduction Fertility, and Development 2011, 23, 3, 403–416.

[6] Rudel, R.; Jim, G.; Cl, E.; Rawsthorne, T. W.; Re, D.; Jim, A.; Rizzo, J.; Nudelman, J. L.; Brody, J. G. Food Packaging and Bisphenol A and Bis(2-Ethylhexyl) Phthalate Exposure: Findings from A Dietary Intervention. Environmental Health Perspectives 2011, 119, 914–920. DOI: 10.1289/ehp.1003170.

[7] Jordakova, I.; Dobiáš, M. Z.; V. O. L. D. R. I. C. H.; Pousta, J. Of Bisphenol A, Bisphenol F, Bisphenol A Diglycidyl Ether and Bisphenol F Diglycidyl Ether Migrated from Food Cans Using Gas Chromatography-Mass Spectrometry,Czech. Journal of Food Sciences 2003), Determination, 21, 85–90. DOI: 10.17211/3481-CJFS.

[8] Llobet, J. M.; Falco, G.; Casas, C.; Teixidó, A.; Domingo, J. L. Concentrations of Arsenic, Cadmium, Mercury, and Lead in Common Foods and Estimated Daily Intake by Children, Adolescents, Adults, and Seniors of Catalonia, Spain. Journal of Agricultural and Food Chemistry 2003, 51, 838–842. DOI: 10.1021/jf020734q.

[9] Bordajandi Luisa, R. J.; Gómez G;Abad, E.; Rivera, J.; Del Mar Fernández-Bastón, M.; Blasco, J.; Mj, G. Survey of Persistent Organochlorine Contaminants (Pcbs,Pcd,Fs, and PAHs), Heavy Metals (Cu, Cd, Zn, Pb, and Hg), and Arsenic in Food Samples from Huelva (Spain): Levels and Health Implications. Journal of Agricultural and Food Chemistry 2004, 52, 992–1001. DOI: 10.1021/jf030453y.

[10] Guo, Y. B.; Feng, H.; Chen, C.; Jia, C.-J.; Xiong, F.; Ying, L. Heavy Metal Concentrations in Soil and Agricultural Products near an Industrial District. Polish Journal of Environmental Studies 2013, 22, 1357–1362.

[11] Sanchez, A. G.; Martos, N. R.; Ballesteros, E. Multiresidue Analysis of Pesticides in Olive Oil by Gel Permeation Chromatography Followed by Gas Chromatography–Tandemmass-Spectrometric Determination. In Analytica Chimica Acta, Elsevier Science, 2006, 558, 53–61.

[12] Payá, P.; Anastassiadès, M.; Mack, D.; Sigalova, I.; Tasdelen, B.; Oliva J;Barba, A. Analysis of Pesticide Residues Using the Quick Easy Cheap Effective Rugged and Safe (Quechers) Pesticide Multi-Residue Method in Combination with Gas and Liquid Chromatography and Tandem Mass Spectrometric Detection. Analytical And Bioanalytical Chemistry 2007, 389, 1697–1714. DOI: 10.1007/s00216-007-1610-7.

[13] Ramesh, A.; Balasubramanian, M. Rapid Preconcentration Method for the Determination of Pyrethroid Insecticides in Vegetable Oils and Butter Fat and Simultaneous Determination by Gas Chromatography–Electron Capture Detection and Gas Chromatography–Mass Spectrometry. The Analyst 1998, 123, 1799–1802. DOI: 10.1039/a803097i.

[14] Gilbert-López, B.; J F, G.-R.; Molina-Díaz, A. Sample Treatment and Determination of Pesticide Residues in Fatty Vegetable Matrices: A Review; Talanta;Elsevier Science; 2006, 79, 109–128.

[15] Barrek, S.; Paisse, O.; Grenier-Loustalot, M. F. Determination of Residual Pesticides in Olive Oil by GC–MS and HPLC–MS after Extraction by Size-Exclusion Chromatography. Analytical and BioAnalytical Chemistry 2003, 376, 355–359. DOI: 10.1007/1997-y.

[16] Pehlivan, E.; Arslan, G.; Gode, F.; Altun, T.; Özcan, M. M. Determination of Some Inorganic Metals in Edible Vegetable Oils by Inductively Coupled Plasma Atomic Emission Spectroscopy (ICP-AES). GRASAS Y ACEITES 2008, 59, 239–244. DOI: 10.3989/gya.2008.v59.s13.

[17] Galtier, O.; Dupuy, Y.; Le Dréau, ; Ollivier;C, D.; Pinatel, ; Kister, J.; Artaud, J. Geographic Origins and Compositions of Virgin Olive Oils Determined by Chemometric Analysis of NIR Spectra. Analytica Chimica Acta 2007, 595, 136–144.

[18] Ansari, R.; Kazi, T. G.; Jamali, M. K.; Arain, M. B.; Wagan, M. D.; Jalbani, N.; Afridi, H. I.; Shah, A. Q. Variation in Theaccumulation of Heavy Metals in Different Verities of Sunflower Seed Oil with the Aid of Multivariate Technique. FoodChemistry 2009, 115, 318–323.

[19] NurAlam;M, M. D.; Chowdhury, A. Z.; Hossain, M. S.; Rahman, M.; Rahman, M. A.; Gan; Md, S. H.; Khalil, I. Detection of Residual Levels and Associated Health Risk of Seven Pesticides in Fresh Eggplant and Tomato Samples from NarayanganjDistrict, Bangladesh. Journal of Chemistry 2015, 243574, 7.

[20] Zheng, N.; Wang, Q.; Zhang, X.; Zheng, D.; Zhang, Z.; Zhang, S. Population Health Risk Due to Dietary Intake of Heavy Metals in the Industrial Area of Huludao City, China. TheScience of the Total Environment. 2007, Volume 387, Issues 1–3, 15 November Pages 96–104. DOI: 10.1016/j.scitotenv.2007.07.044.

https://www.agilent.com/cs/library/selecionguide/public/5990-8590EN.pdf

https://iupac.org/
[23] https://en.wikipedia.org/wiki/Elution
[24] https://www.ncbi.nlm.nih.gov/books/NBK231554/
[25] Mnif, W.; HadiHassine, A. I.; Bouaziz, A.; Bartegi, A.; Thomas, O.; Roig, B. Effect of Endocrine Disruptor Pesticides: A Review. International Journal Environment Researcher Public Health 2011, 8(6), 2265–2303. DOI: 10.3390/ijerph8062265.
[26] Robert, Kavlocka; Boekelheide, K.; Chapin, R.; Cunningham, M.; Faustman, E.; Foster, P.; Golub, M.; Henderson, R.; Hinberg, I.; Little, R.; et al., NTP Center for the Evaluation of Risks to Human Reproduction: Phthalates Expert Panel Report on the Reproductive and Developmental Toxicity of Di-N-Butyl Phthalate, Reproductive Toxicology 16 (2002) 489–527.
[27] Eskenazi, B.; Bradman, A.; Castorina, R. Exposures of Children to Organophosphate Pesticides and Their Potential Adverse Health Effects. Environmental Health Perspectives. 107, 3, June 1999, pp 409–419.
[28] http://www.sciencedirect.com/topics/page/Xenoestrogen
[29] Boening, D. W.; Ecological E Acts, Transport, and Fate of Mercury: A General Review. Chemosphere 2000, 40, 1335–1351.
[30] https://en.wikipedia.org/wiki/Cadmium_poisoning
[31] http://www.medicinenet.com/arsenic_poisoning/article.htm
[32] https://en.wikipedia.org/wiki/Lead_poisoning
[33] Pavesi, A. A.; Vicente, E.; Zanes, F. R. P.; De Figueiredo, T. M. C. Estimate of Dietary Intake of Chloropropanols (3-MCPD and 1,3-DCP) and Health Risk Assessment. Food Science and Technology (Campinas) 2013, 33(Suppl. 1), 125–133. DOI: 10.1590/S0101-20612013000500019.
[34] O’Connor, J. C.; Chapin, R. E. Critical Evaluation of Observed Adverse Effects of Endocrine Active Substances on Reproduction and Development, the Immune System, and the Nervous System. Pure Applications Chemical. 2009–2123, 2003, 75, 11–12, Nos 2099–2123.