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PHYSICOCHEMICAL AND MINERAL PROPERTIES OF GUAVIRA (*Campomanesia adamantium* (Cambess.) O. Berg.) FRUIT PULP AND SUNFLOWER (*Helianthus annuus* L.) SEED OIL OBTAINED IN THE CERRADO BIOME

PROPRIEDADES FÍSICO-QUÍMICAS E MINERAIS DA POLPA DO FRUTO DA GUAVIRA (*Campomanesia adamantium* (Cambess.) O. Berg.) E ÓLEO DA SEMENTE DE GIRASSOL (*Helianthus annuus* L.) OBTIDOS NO BIOMA CERRADO

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Campo Grande
MS – Brazil
2023

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To my family**:**

Johnson, Windaus, Philips, Calvin, Judith, Filipe, Julieta, Teresa, Elias, Fátima, Rosa and *in memorium*: Johane, Fátima, Zarina and Joseph.

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ABSTRACT

Cerrado biome is a second major area with 22% of extension, localized in central region of Brazil, which Mato Grosso do Sul state is found with 61% of this biome. Mato Grosso do Sul state is a home of the approximately 4,000 of native Angiosperm species catalogued. Among them stand out pequi, baru, guavira, cajuzinho, etc., because some of them are source of fruits, seeds, nuts, with high bioactive compound and nutritional content. Moreover, species as bocaiuva, guavira, baru provide vegetable oil of high nutritional quality for human consumption. In addition, the soils of the Cerrado region are arable for several crops such as soybean, sunflower, corn, peanut, coffee, cotton sugarcane, and others, which are used for human and animal consumption. Therefore, the Cerrado natural soil is characterized by high amount of aluminium (Al), and with low contents of phosphorus (P), zinc (Zn), iron (Fe), vanadium (V), chromium (Cr), barium (Ba), lead (Pb), nickel (Ni), copper (Cu), molybdenum (Mo) and cadmium (Cd). For this reason, biosolid, manure, fertilizer, and pesticides are massively used for Cerrado soil correction that are rich in minerals, which can contaminate soils, crops and native plants. Although, the consumption of food that are source of minerals (macro– and microelements) is largely recommended for health promotion and disease prevention, however high amount of these minerals can be toxic and hazard for several organs functions. Thus, two chapters are described in this work, which the first chapter present studies conducted with pulp of *Campomanesia adamantium* (guavira) fruit obtained from the roadside (500 m), bush (1000 m) and farm-margin (3000 m) measured from the road, mineral amount were potassium, lead, phosphorus, arsenic, selenium, iron, molybdenum, zinc, cobalt, nickel, manganese, and chromium were quantified and compared with Dietary Reference Intakes (DRI), the Food Agriculture Organization of the United Nations (FAO) and World Health Organization (WHO) and the Food and Drug Administration (FDA) established limits. The second chapter report studies realized with sunflower oil obtained from dried hull seeds sample cold-press extracted using a domestic machine, which were evaluated the fatty acids composition, physicochemical and optical properties, thermal and oxidative stability were compared with previous studies, however the mineral elements Mg, Cr, Mn, Fe, Co, Ni, Cu, Zn, Cd, Al, Pb, As, and Se were quantified by inductively coupled plasma optical emission spectroscopy (ICP OES) and compared with DRI/AI, and FAO/WHO parameters.

Keywords: Cerrado, vegetable, pulp; seed, oil, mineral, non-carcinogenic indices.

RESUMO

Cerrado é o segundo maior bioma com a extensão de 22% depois da Amazona, localizada na região central do Brasil, na qual no estado de Mato Grosso do Sul compreende 61% deste bioma. Estado de Mato Grosso do Sul abriga aprox. 4 mil espécies nativas de Angiosperma catalogadas. Dentre elas destacam-se pequi, baru, guavira, cajuzinho, etc., pois algumas delas fornecem frutos, sementes, amêndoas com alto teor de substâncias bioativas e nutricionais. Além disso, espécies como bocaiuva, guavira, pequi, baru fornecem óleo de alta qualidade nutricional para o consumo humano. Em adição, os solos do Cerrado são aráveis para culturas como soja, girassol, milho, amendoim, café, algodão, cana-de-açúcar, e outras, usadas na alimentação humana e animal. Entretanto, o solo natural do Cerrado é caracterizado pela alta concentração do aluminium (Al), e com baixos teores de fósforo (P), zinco (Zn), ferro (Fe), vanádio (V), cromo (Cr), bário (Ba), chumbo (Pb), níquel (Ni), cobre (Cu), molibdênio (Mo) e cadmio (Cd). Por isso, biossólidos, adubos, fertilizantes e agrotóxicos são massivamente utilizados para a correção destes solos, que ricos em minerais, que podem contaminar solos, e plantas nativas e cultivadas. Embora o consumo de alimentos ricos em minerais (macro– e microelementos) seja amplamente recomendado para promoção da saúde e prevenção de doenças. No entanto, a alta quantidade desses minerais pode ser tóxico e prejudicial para funções vitais de vários órgãos. Assim, dois capítulos são descritos neste trabalho, na qual o primeiro apresenta estudos realizados com a polpa do fruto de *Campomanesia adamantium* (guavira) obtido a partir da beira da estrada (500 m), mata (1000 m) e margem da fazenda (3000 m). Os minerais (K, Pb, P, As, Se, Fe, Mo, Zn, Co, Ni, Mn e Cr) foram quantificados usando espectrometria de emissão atômica por plasma acoplado indutivamente (ICP OES) e comparados com os parâmetros estabelecidos da Consumo Dietético de Referência (DRI), Organização das Nações Unidas para a Alimentação e a Agricultura (FAO) e a Organização Mundial da Saúde (OMS), e a Administração para Alimentos e Drogas (FDA). O segundo capítulo aborda estudos realizados com óleo de girassol obtido a partir de sementes secas com cascas extraídas por prensagem a frio em uma máquina doméstica, onde foram avaliadas a composição de ácidos graxos, propriedades físico-químicas e ópticas, estabilidade térmica e oxidativa, e foram comparadas com estudos da literatura, porém minerais: Mg, Cr, Mn, Fe, Co, Ni, Cu, Zn, Cd, Al, Pb, As e Se foram quantificados por espectroscopia de emissão óptica de plasma acoplado indutivamente (ICP OES) e foram comparados com os parâmetros da DRI e FAO/OMS.

Palavras-chaves: Cerrado, vegetal, polpa; semente, óleo, mineral, índice não-carcinogênico.

SUMMARY

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GENERAL INTRODUCTION

Brazilian Cerrado is a second largest biogeographic area after Amazon, comprise 22% of extension, situated in central region of Brazil (BRASIL, 2021), which correspond 61% (214.779 km^2) of the territory of Mato Grosso do Sul state (Silva et al., 2010). In this region, approximately 12000 Angiosperm species were catalogued, corresponding 30.48% (3,657) to Mato Grosso do Sul state (BFG, 2015). Some of them are popularly used as edible plants, which provide fruits and seeds with high bioactive compounds and nutritional content, e.g: pequi (*Caryocar brasiliense* Camb.), baru (*Dipteryx alata* Vog.), guavira (*Campomanesia* spp.), cajuzinho (*Anacardium humile* St. Hil.) (Bailão et al., 2015; Bortolotto et al., 2018). Moreover, others are potential vegetable oil producer like bocaiuva (*Acrocomia aculeate* (Jacq.) Lodd. ex Mart.), guavira (*Campomanesia adamantium* Vog.), baru (*Dipteryx alata* Vog.), pequi (*Caryocar brasiliense* Camb.) (Bortolotto et al., 2017; Machate et al., 2020).

On the other hand, Cerrado region is largely used for several edible crops production such as sunflower, soybean, peanut, coffee, corn, cotton, sugarcane, and etc. (Ferraz-Almeida and da Mota, 2021; Spera, 2017).

Therefore, all plants above mentioned are utilized for human consumption *in natura* and derivative obtain as pulp, flour, nuts, oils, bran, fibre, which are used as food for human and animal (Bortolotto et al., 2017; Wilkinson and Lee, 2018).

Although, the Cerrado natural soil is characteracted by high amount of aluminium (Al) and low quantity of phosphorus (P) and zinc (Zn), iron (Fe), vanadium (V), chromium (Cr), barium (Ba), lead (Pb), nickel (Ni), copper (Cu), molybdenum (Mo), cadmium (Cd), elements (Macedo et al., 2014; Brito et al., 2020). Due to the Cerrado soil topography, is largely utilized biosolid, fertilizer and pesticide to ameliorate soil and crop production (Souza and Lobato, 2004; Nogueira et al., 2013). In addition, biosolid, manure, fertilizer and pesticides are composed by several metals such as potassium (K), calcium (Ca), magnesium (Mg), Fe, Cu, Cr, Cd, cobalt (Co), manganese (Mn), Pb, Ni, mercury (Hg), Mo, Zn, metalloids (arsenic (As) and selenium (Se), non-metals (nitrogen (N), sulphur (S), P (Srivastava et al., 2016; Tóth et al., 2016; Chandra and Kumar, 2017; Sharma et al., 2017; Srivastava et al., 2017; Woldetsadik et al., 2017). The higher amount of metals and metalloids are reported in edible vegetables, fruits, and oils of conventional plants (Zhu et al., 2011; Elbagermi et al., 2012; Guerra et al., 2012; Farzin et al., 2014).

Therefore, As, Pb and Cr chemical elements were quantified high in soot from petroleum, dust of the roads, brake lining and tire wear and in soil and plants growing near to a road (Adamiec et al., 2016; Nwaedozie and Nyan, 2018; Kuklová et al., 2022). In addition, As, Pb and Cr were

observed high in fertilizer used to improve agricultural soils as commercial phosphate fertilizers (Gonçalves Jr et al., 2014; Jayasumana et al. 2015; Jami Al-Ahmadi et al. 2018; Guilherme etal., 2020). Moreover, As was quantified majorly in Glyphosate-based herbicides for example R Weather Max, Clinic EV, R3+, Radical Tech+. Thus, As, Cr and Pb were quantified high in fungicides (Folpan, Eyetak, Pictor and Teldor), herbicides (Matin and Starane) and insecticides (pyrinex) formulations, which appeared 5 to 100 times compared with permitted limits and due to their potential bioaccumulation ability can be hazardous to the environment and living things, including humans (Defarge et al., 2018, Seralani and Jungers, 2020).

Beyond, through wind, rain, and water currents chemical elements can reach areas not used for agriculture propose, which can modify macro– and microelements composition and concentration in edible plants, absorbed by roots to term, leaves, and fruits (Srivastava et al., 2017; Antoniadis et al., 2019; Margenat et al., 2019; Rai et al., 2019).

The consumption of food source of macro– and microelements is broadly recommended for maintaining the consumers' better health (Jaishankar et al., 2014). However, food intake with higher amount of these metals and metalloids are correlated with brain, lung, kidney, liver dysfunctions, Parkinson disease, Alzheimer disease, cardiovascular diseases, and cancers (Järup, 2003; Jaishankar et al., 2014).

Although several studies with edible native such as mangaba (*Hancornia speciosa* Gomes) pulp (Cardoso et al., 2014), *Campomanesia* spp. fruits and pulp (Verruck et al., 2021; Cardozo et al., 2018), *C. adamantium* seed oil (Machate et al., 2020), bocaiuva pulp (Nunes et al., 2020), canjica (*Byrsonima cydoniifolia* A. Juss) fruits (Marcelino et al., 2019), baru nut (Oliveira-Alves et al., 2020), buriti (*Mauritia flexuosa* L.f.) oil (Aquino et al., 2012) and cultivated plants as soybean fruit and its by-products (Nogueira-de-Almeida et al., 2020), sunflower oil (Castro and Leite, 2018) and noni (*Morinda citrifolia* L.) (Inada et al., 2020) of the Cerrado biome demonstrated health benefits. However, there are few studies in the literature on heavy metals occurrence in the Cerrado native and cultivated edible plants (Lima et al., 2017; Melo et al., 2019; Rosa et al.; 2020; Germano et al., 2021; Rosa et al., 2021).

Thus, justifies for the present study to perform the quantification of macro– and microelements in *C. adamantium* pulp (native fruit plant) and compared with DRIs/AI* and FAO/WHO parameters, and non–carcinogenic human health risk assessment. Additionally, characterized fatty acids composition and nutritional quality, and evaluated physicochemical and optical proprieties, and thermal and oxidative qualities of the sunflower oil (cultivar oil plant) extracted using a cold-press domestic machine.

This work is presented in two chapters describing *C. adamantium* pulp, and sunflower (*Helianthus* annuus) oil, which their articles were published and available in the literature, and annexed in this thesis, respectively as presented:

1. **David Johane Machate**, Elaine S. de Pádua Melo, Daniela G. Arakaki, Rita de Cássia Avellaneda Guimarães, Priscila Aiko Hiane, Danielle Bogo, Arnildo Pott and Valter Aragão do Nascimento. High concentration of heavy metal and metalloid levels in edible *Campomanesia adamantium* pulp from anthropic areas. International Journal of Environmental Research and Public Health. 2021, 18(11), 5503. https://doi.org/10.3390/ijerph18115503

2. **David Johane Machate**, Elaine S. P. Melo, Lincoln Carlos Silva de Oliveira, Danielle Bogo, Flávio S. Michels, Arnildo Pott, Leandro F. Cavalheiro, Rita de Cássia Avellaneda Guimarães ... Valter Aragão do Nascimento. Oxidative stability and elemental analysis of sunflower (*Helianthus annuus*) edible oil produced in Brazil using a domestic extraction machine. Frontiers in Nutrition 2022, 9, 977813. https://doi.org/10.3389/fnut.2022.977813

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CHAPTER I

Macro- and microelements in *Campomanesia adamantium* (Cambess.) O. Berg fruit pulp

1.1. ABSTRACT

This study aimed to quantify the extent of heavy metal, non-metal and metalloid levels in the *Campomanesia adamantium* pulp obtained from an area crossed by road experiencing high large vehicle traffic and intensive agriculture modern farming, to monitor the health risks associated with pulp consumption by humans. For this purpose, in three spots located within this area, ripe fruits were collected on the roadside (500 m), bush (1000 m) and farm-margin (3000 m). Pulp samples were digested by microwave-assisted equipment, and chemical elements were quantified by ICP OES. The concentrations of K, Pb, Se, Fe, Mo, Zn, Co, Ni and Mn in the pulp collected in roadside/bush points showed statistical differences ($p < 0.05$). The heavy metals and metalloid concentrations that exceeded FAO/WHO standards were ordered Pb > As > Mo > Co > Ni > Mn > Cr. Therefore, among these metalloid and heavy metals, As, Pb and Cr were found to be higher in farm-margin > roadside > bush (1.5×10^{-3}, 1.1×10^{-3} and 6.2×10^{-4}), respectively. Therefore, As is the most important metalloid with higher levels in farm-margin, roadside and bush (1.5×10^{-3}, 1.0×10^{-3} and $6.0 \times 10^{-4} > 10^{-6} – 10^{-4}$ and 3.33, 2.30 and 1.34 > 1), respectively, to total cancer risk and hazard quotient, if 10 g daily of pulp are consumed.

Keywords: Cerrado, Myrtaceae, edible fruit, farm-margin, roadside, macro- and microelements, health risk

CAPÍTULO I

Macro- e microelementos na polpa do fruto de *Campomanesia adamantium* (Cambess.) O. Berg

RESUMO

Este estudo objetivou quantificar as concentrações de metais pesados, não metais e metaloides na polpa do fruto de *Campomanesia adamantium* coletado em uma área localizada entre uma estrada com alto tráfego de veículos pesados e uma fazenda de agricultura intensiva moderna, para monitorar os riscos à saúde associados ao consumo desta polpa por humanos. Para esta proposta, os frutos foram coletados em três pontos localizados nesta área, beira da estrada (500 m), mata (1000 m) e na margem da fazenda (3000 m). As amostras da polpa foram digeridas por um equipamento de micro-ondas assistido e os elementos químicos foram quantificados por um ICP OES. As concentrações de K, Pb, Se, Fe, Mo, Zn, Co, Ni e Mn nas polpas coletadas mostraram diferenças estatísticas ($p < 0,05$). A concentração de metais pesados e metaloides que excederam padrões da FAO/OMS foram ordenados em Pb > As > Mo > Co > Ni > Mn > Cr. Entretanto, dentre estes metaloides e metais pesados, As, Pb and Cr foram encontradas maiores concentrações na margem da fazenda > beira da estrada > mata ($1,5 \times 10^{-3}$; $1,1\times 10^{-3}$ e $6,2 \times 10^{-4}$), respectivamente. Portanto, As é o metaloide mais importante com maiores níveis de concentração na margem da fazenda, beira da estrada e na mata ($1,5 \times 10^{-3}$; $1,0 \times 10^{-3}$ e $6,0 \times 10^{-4} > 10^{-6} – 10^{-4}$ e 3,33; 2,30 e 1,34 > 1), respectivamente, para o risco total de câncer e quociente de risco, se for consumido diariamente 10 g desta polpa.

Palavras-chaves: Cerrado, Myrtaceae, fruto comestível, margem da fazenda, beira da estrada, macro– e microelementos, risco à saúde.

1.2. INTRODUCTION

1.2.1. *Campomanesia adamantium* (Cambess.) O. Berg

The genera *Campomanesia* Ruiz et Pav is circumscribed to Myrtaceae Juss. family composed by trees and shrubs (0.3 – 20 m height), distributed from Northeast of Argentina to Trinidad and Tobago (Caribbean), from Brazilian coast until Andes in Peru, Equator and Colombia (Landrum, 1982). There are known 80 species of *Campomanesia* (GBIF, 2018), which 60 were identified in Brazil and 25 are endemic (Flora do Brasil, 28. 04. 2022). In Brazil, *Campomanesia* species are popularly known by "guavira", "gabiroba", "guariroba", "guabiroba", "guabiroba-do-campo" (Porto and Gulias, 2010). The floristic studies in Mato Grosso do Sul state described the occurrence of six species: *Campomanesia aurea* O. Berg, *C. xanthocarpa* (Mart.) O. Berg, *C. guazumifolia* (Cambess.) O. Berg, *C. sessiliflora* (O. Berg) Mattos, *C. pubescens* (Mart. Ex DC.), and *C. adamantium* (Cambess.) O. Berg (Proença et al., 2018).

Campomanesia adamantium species present 0.5 – 2 m height; leaves glabrous or scarce trichomes, blade elliptic, oblong, or obovate, apices acute to acuminate, base acute or obtuse, lateral nerves 10 – 20; flower with glabrous, lobules more length than breadth, interior or margin with trichomes; ovary with 5 – 9 locules; fruits broadly ovoid, glabrous and smooth (Lima et al., 2011). To *C. adamantium* flowering occurs from August to October and its fruiting start in November to December (Vieira et al., 2016).

Campomanesia adamantium is largely used in popular medicine in treatment inflammatory diseases, obesity, diarrhoeas, vomits, and hypertension (Cardoso et al., 2010). On the other hand, its fruits are fresh consumed, and used to obtain home liqueurs, juice, sweets, jelly, ice creams and beverage industry (Leão-Araújo et al., 2019).

1.2.2. Chemical compounds

Studies have reported the occurrence of several chemical compounds in *C. adamantium* such as lipids, proteins, carbohydrates, fibres, ascorbic acid (vitamin C), riboflavin (vitamin B2) were described in fruits (Valillo et al., 2006). Additionally, polyphenols compounds were reported in root, leaf, fruit, pulp such as phenolic acid: gallic and ellagic acids, flavonoids: flavonols (campeferol, quercetine, myricetine, flavanone (estrobopinine, 5,7-dimethoxy-6-methylflavanone, demetoximateucinol, 5-hydroxi-7-metoxy-8metilflavanone, prociadine, prodelfinidine, galocatechine, epigalocatechine, catechine, epicatequine and chalcone (aurentiacine A, dimetilchalcone, cardamonine, champanone C and D) (Pascoal et al., 2014; Espindola et al., 2016;

Campos et al., 2017; Lima e Silva et al., 2018; Villas Boas et al., 2018). As well as, palmitic and oleic are the primarily fatty acids reported in oil seeds (Machate et al., 2020). Moreover, terpenes or terpenoids found in leaf, flower, peel and seeds more represented by verbena, B-funebrene, limonene, sabinene, a-thujene and methyl salicylate (Viscardi et al., 2017; Sá et al., 2018). As well as, macroelements: sodium (Na), potassium (K), and calcium (Ca) and microelements: magnesium (Mg), phosphorus (P), iron (Fe), zinc (Zn), nickel (Ni), manganese (Mn), cobalt (Co), copper (Cu), molybdenum (Mo), chromium (Cr), silicon (Si), and aluminium (Al) were quantified in peel, pulp and seeds (Lima et al., 2017).

Thus, the chemical compounds, and macro- and microelements reported in roots, leaves, fruits (peel, pulp and seed) make positively activities of their extracts as antibacterial, antifungal (Pavan et al., 2009; Coutinho et al., 2009; Sá et al., 2018), anticancer (Pascoal et al., 2014; Campos et al., 2017; Lima e Silva et al., 2018), anti-platelet aggregation, anticyclooxigenase (Lescano et al., 2018), antidiarrheal (Lescano et al., 2016), DNA damage, apoptosis (Martello et al., 2016), antioxidant, hepatoprotective (Fernandes et al., 2015), antihyperlipidemic in vitro studies (Espindola et al., 2016). As well as, anti-inflammatory, leukocyte antimigration, antioedema, antinociceptive, neurogenic antipain, antihyperalgesic, and antidepressive effects in rodents (Souza et al., 2014; Viscardi e al., 2017).

Therefore, the health benefits above reported justify the importance and largely use of the *C. adamantium* in food, and popular medicines, as well as broadly interest in nutraceutical research and derivative production by industry.

1.3. OBJECTIVES

1.3.1. General objective

- Quantify macro– and microelements in *Campomanesia adamantium* fruit pulp collected in three spots from the roadside (500 m) to bush (1000 m) and farm-margin (3000 m), marked by intense anthropogenic activities.

1.3.2. Specific objectives

- Quantify mineral composition (macro- and microelements: potassium (K), lead (Pb), phosphorus (P), arsenic (As), selenium (Se), iron (Fe), molybdenum (Mo), zinc (Zn), cobalt (Co), nickel (Ni), manganese (Mn), and chromium (Cr);

- Compare the macro- and microelements pulp amount with recommended tolerable maximum intake levels established by Dietary Reference Intakes for children aged (4 – 8 years, adults and pregnancy (31 – 50 years), and the Food Agriculture Organization of the United Nations (FAO) and World Health Organization (WHO) parameters for human intake;

- Calculate the non-carcinogenic risk by the health risk assessment compared with recommended by WHO parameter for edible fruits and vegetables.

1.4. MATERIALS AND METHODS

1.4.1. Collection and sample preparation

The *Campomanesia adamantium* ripe fruits were collected in twenty-one different points, separated from each other by 20 m. the fruits were mixed according to the collected distance from the roadside (500 m) to the bush (1000 m) and farm-margin (3000 m) in Campo Grande, Mato Grosso do Sul state, Brazil, 20º46'34.208'' S, 54º10'28.567'' in November 2019 (Fig. 1.1.).

Figure 1.1. *Campomanesia adamantium* fruit collection. **(a)** – ripe fruits; **(b)** – spots located between the state road MS-040 with high large vehicle traffic and intensive modern agriculture in Campo Grande – Mato Grosso do Sul state, Brazil. 1. roadside = 500 m; 2. bush = 1000 m; 3. farm-margin = 3000 m.

Manually, the pulp was separated from the peel and seed, immediately dried in an air circulation oven at 40 ºC for 48 h. The dried pulp was milled using mortar and pestle and sieved to obtain the refined powder, placed into an amber and hermetic glass bottle and frozen at –20 ºC for further analyses. The *C. adamantium* was registered in System of Genetic Resource

Management and Associated Traditional Knowledge (SisGen) of the Ministry of the Environment Brazil (registration number A7716EC).

1.4.2. Microwave-assisted digestion procedure

The pulp samples (100 g) were weighted according to Lima et al., (2016) and prepared as described: 0.5 g sample plus 5 mL HNO_3 (65% Merck, Darmstadt, Germany) and 3 mL H_2O_2 (35% Merck, Darmstadt, Germany) were individually placed into PTFE bottles of the DAP 60 type (Berghof). The mixture was allowed to remain in the open air for 10 min predigestion and then digested using a microwave digestion system (Speedwave four®, Berghof, Germany). After the microwave system's digestion procedure, the samples were transferred from the vessels to 50 mL falcon vessels and which were then filled to 30 mL with ultrapure water (conductivity 18.2 MΩcm (Millipore), Biocel, Germany) (Fig. 1.2.).

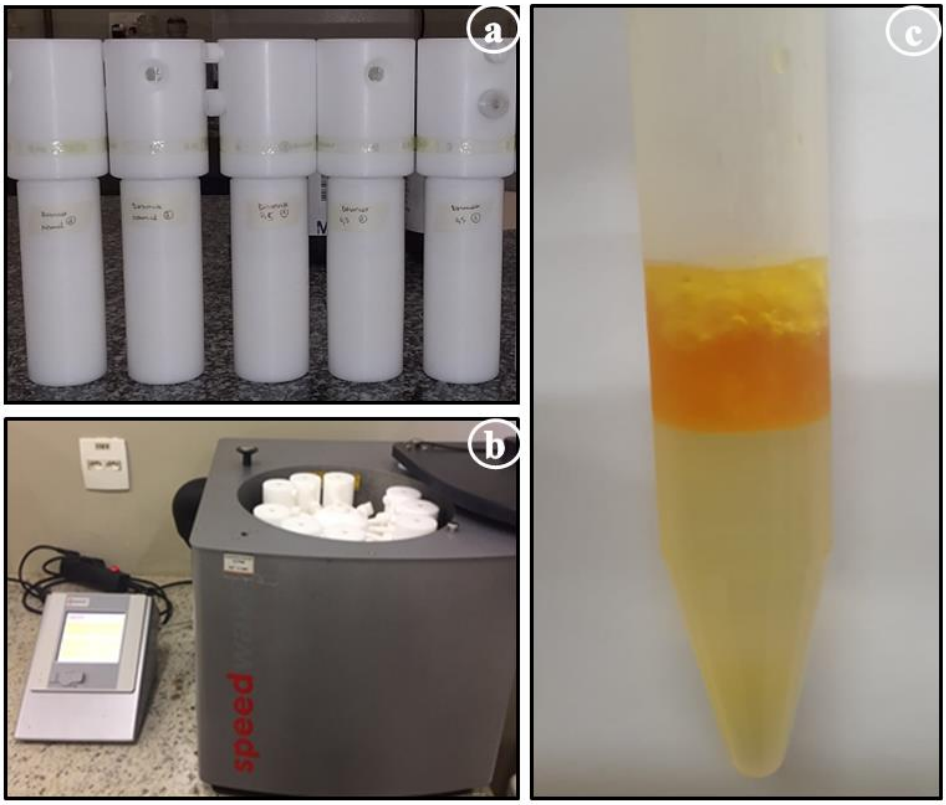

Figure 1.2. Sample digestion system. **(a)** – DAp60 Teflon tubes; **(b)** – close system microwave; **(c)** – falcon vessels.

The samples were digested in the microwave system according to the schedule shown in Table 1.1. All digestion analysis steps were performed in triplicate.

Table 1.1. Microwave digestion parameters.

	Steps			
	1	**2**	**3**	**4**
Power (W)	1305	1305	0	0
Temperature ºC)	170	200	50	50
Ramp time (min)	1	1	1	1
Hold time (min)	10	15	10	1
Pressure (Bar)	35	35	0	0

1.4.3. ICP OES elemental analysis

Chemical elements were quantified using the inductively coupled plasma optical emission spectroscopy (ICP OES) (Thermo Fischer Scientific, Bremen, Germany, iCAP 6300 Duo) technique (Fig. 1.3.).

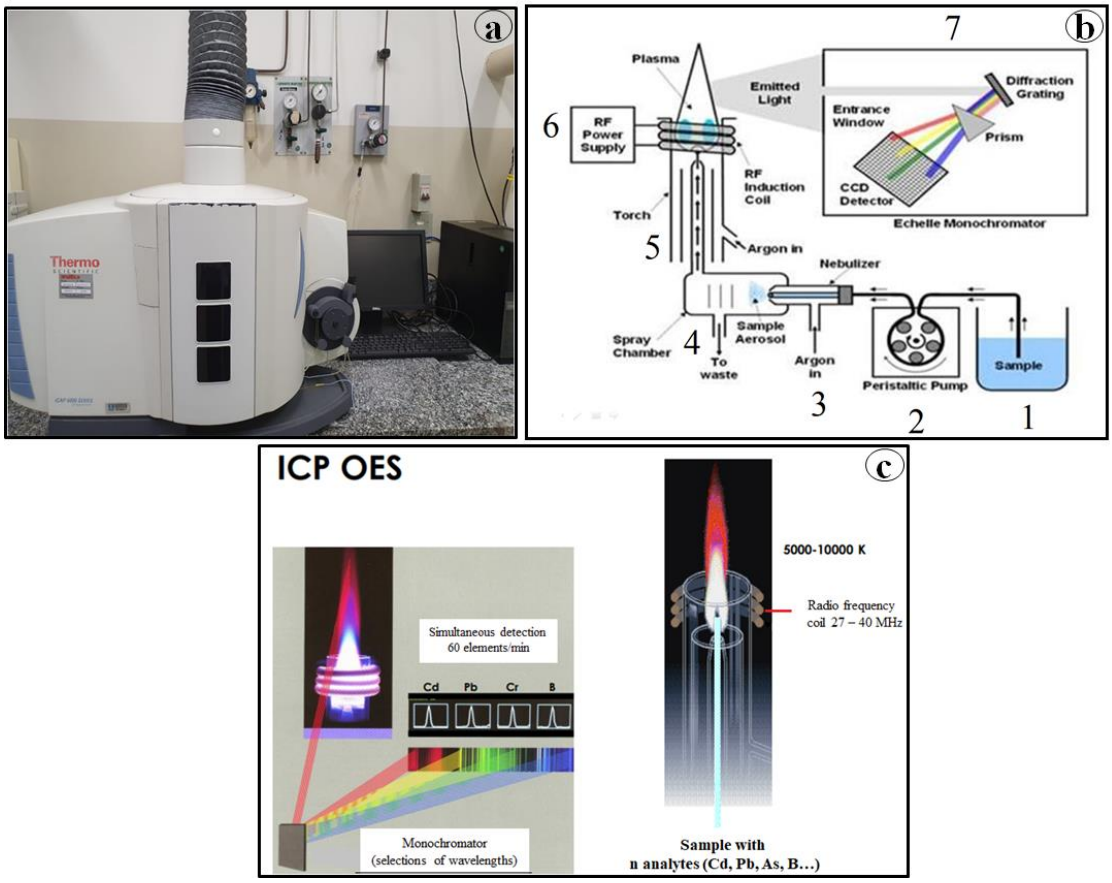

Figure 1.3. Inductively coupled plasma optical emission spectroscopy (ICP OES). **(a)** – apparatus; **(b)** – schematic setup; (**c**) – detail of 6 and . The images **b** and **c** sourced in: chrome-extension://efaidnbmnnnibpcajpcglclefindmkaj/https://edisciplinas.usp.br/pluginfile.php/4312246/mod_resource/content/2/Aula%20-%20ICP%20OES.pdf

The selected emission lines (wavelength in nm) for determining elements in pulp and operating conditions of ICP OES are summarized in Table 1.2.

Table 1.2. Instrumental analytical conditions for the ICP OES of element analysis.

Parameters	Setting
RF power (W)	1250
Sample flow rate (L mn^{-1})	0.45
Plasma gas flow rate (L mn^{-1})	12
Integration time (s)	5
Stabilization time (s)	20
Pressure of nebulization (psi)	20
Plasm view	Axial
Gas view	Air
Analytical wavelength (nm)	Fe (259.940), Ni (231.604), Co (228.616), Cr (267.716), As (193.759), Pb (214.441), Mo (202.030), Mn (257.610), P (177.595), K (766.490), Zn (213.856), Se (196.090)

1.4.4. Calibration curves

For the ICP OES, standard solutions for analytical calibration were prepared by diluting a standard multiple-element stock solution containing 1000 mg/L of the Al, As, Ca, Cd, Co, Cr, Cu, Fe, Mg, Mn, Mo, Na, Ni, P, S, V, Se, and Zn from SpecSol (SpecSol, Quimlab, Brazil). For each element detected, the limit of detection (LOD) of 0.0002 – 0.003 (mg/L), the limit of quantification (LOQ) of 0.006 – 0.01 (mg/L) and the coefficient of determination (R^2) of 0.995 – 0.9998 were determined. One bank and seven calibration curves were generated using the following concentrations: 0.01, 0.02, 0.05, 0.2, 1.0, 2.0 and 5.0 mg/L the element standard. All experiments were carried out in triplicate. The detection limit (LOD) was calculated as three times the standard deviation of the blank signal (B) expressed in concentration divided by the slope of the analytical curve (AC): LOD = 3*B/AC, and the limit the slope of the analytical curve: LOQ = 10*B/AC (Thompson et al., 2002).

An addition/recovery test for the elements under study was performed in a pulp sample by spiking 0.5 mg/L of each analyte. The method had a recovery internal of 80% – 110 for the spike 0.5 mg/L, which was found to be between 70% and 120% to the established limit proposed by the Union of Pure and Applied Chemistry (IUPAC) and Association of Official Analytical Chemists (AOAC, 2021; Antoniadis et al., 2019).

1.4.5. Human health risk assessment

The results of the concentrations of the chemical elements were compared with recommended intake standards of the RDA/AI, UL, FAO/WHO, US EPA and hazard quotient. The human risk for a non-carcinogenic was calculated following the equation adopted by Liang et

al., 2017. Cancer risk was the probability of an individual developing any cancer over a lifetime, during the daily doses exposure to 70 years; the chronic daily intake dose (CDI) of carcinogenic elements (g kg^{-1} day^{-1}); and slope factor (SF) was the carcinogenicity (mg kg^{-1} day^{-1}). The SFs of As, Cr and Pb are 1.5, 0.5 and 0.0085 mg kg^{-1} day^{-1}, respectively, following Equation (1.1):

$$\text{Cancer Risk} = \text{CDI} \times \text{SF} \tag{1.1}$$

The cancer risk is a sum of individual variety carcinogenic elements risk in different exposure pathways, which is the total cancer risk ®. In agreement with US EPA (1989), the value of acceptable or tolerable cancer risk ranges from 10^{-6} to 10^{-4} is considered unacceptable.

The human health risk of heavy metal intake was evaluated based on the chronic daily intake dose (CDI, mg kg^{-1} day^{-1}) for a chemical contaminant in the pulp over the exposure period and the pulp intake quantity. CDI calculated using the following Equation (1.2):

$$\text{CDI}_{pulp} = \frac{\text{C}_{pulp} \times \text{IR}_{pulp} \times \text{EF} \times \text{ED}}{\text{BW} \times \text{AT}} \tag{1.2}$$

Where CDI_{pulp} – chronic daily pulp intake dose; C_{pulp} – concentration of chemical element content in the pulp; IR_{pulp} – ingestion rate (mg day^{-1}); EF – exposure frequency (90 days available/year); ED – exposure duration (life expectancy = 70 years); BW – body weight; and AT – average time (ED × 365 day). The adult's body weight, approximately 70 kg, and the average daily pulp consumption was 10 g day^{-1}. The risk to human health by the intake of heavy metal – contaminated pulp was measured using a hazard quotient (HQ), which is a ratio of CDI and chronic oral reference dose (RfD), determined by the following Equation (1.3):

$$\text{HQ} = \frac{\text{CDI}}{\text{RfD}} \tag{1.3}$$

The RfD values for the risk calculation were established by the Joint Food and Agriculture Organization/World Health Organization Expert Committee on Food Additives (JECFA/WHO, 2003) and the United States Environmental Protection Agency (US EPA IRIS, 2021). The RfD values for the elements were established: K = not available; Pb = 0.004 mg kg^{-1} bw day^{-1}; P = not available; As = 0.0003 mg kg^{-1} bw day^{-1}; Se = not available; Fe = 0.7 mg kg^{-1} bw day^{-1}; Mo = 0.005 mg kg^{-1} bw day^{-1}; Zn = 0.3 mg kg^{-1} bw day^{-1}; Co = 0.03 mg kg^{-1} bw day^{-1}; Ni = 0.02 mg kg^{-1} bw day^{-1}; Mn = 0.14 mg kg^{-1} bw day^{-1}; and Cr = 0.003 mg kg^{-1} bw day^{-1}. (US EPA IRIS, 2021). As shown in Equation (3), a toxic risk is considered to occur if HQ > 1, whereas HQ < 1 represents a negligible hazard (adverse non-carcinogenic effects).

1.4.6. Statistical Analysis

The data were analysed by one-way ANOVA using the GraphPad Prism software version 8.0 for Mac (GraphPad Software, San Diego, CA, USA). The significance of the differences between the means for the individual element level was considered at $P < 0.05$.

1.5. RESULTS AND DISCUSSION

This part was constituted of two sections: Section 1.5.1. presented data on the concentration of the chemical elements obtained in pulp collection in roadside, bush and farm-margin, and the comparison of these concentrations with other published studies. In Section 1.5.2, data of the type of chemical elements quantified for each site used to calculate EDI and HQ values.

1.5.1. The chemical elements concentration in pulp collected in three different sites

Twelve chemical elements were found in *C. adamantium* pulp collected in three different sites from the road: roadside (500 m), bush (1000 m) and farm-margin (3000 m) (Table 1.3.).

Table 1.3. Campomanesia adamantium pulp collection in three different sites from the road: roadside (500 m); bush (1000 m); and farm-margin (3000 m), quantified by ICP OES (mg/100g g ± SD) compared with nutritional recommendations for adult, pregnancy and children by DRIs: RDA/AI*; UL and FAO/WHO.

Elements	Roadside ($mg100g^{-1}$)	Bush ($mg100g^{-1}$)	Farm-margin ($mg100g^{-1}$)	Male 31–50 y RDA/AI* ($mg100g^{-1}$)	Female 31–50 y RDA/AI* ($mg100g^{-1}$)	Male/Female 31–50 y UL ($mg100g^{-1}$)	Pregnancy 31–50 y		Children 4–8 y		FAO/WHO ($mg\ day^{-1}$)
							RDA/AI* ($mg100g^{-1}$)	UL ($mg100g^{-1}$)	RDA/AI* ($mg100g^{-1}$)	UL ($mg100g^{-1}$)	
K	33.02±0.01	31.02±0.01	58.01±1.34	4700	4700	ND	4700	ND	4700	ND	3510 (WHO, 2012)
Pb	5.36±0.02	7.02±0.01	6.85±1.05	ND	ND	ND	ND	ND	ND	ND	0.02 (FAO/WHO, 2011)
P	3.24±0.02	3.04±0.02	5.24±0.80	700	700	4000	700	3500	500	3000	700 (WHO, 2012)
As	1.96±0.04	1.14±0.03	2.84±0.52	ND	ND	ND	ND	ND	ND	ND	0.01 (FAO/WHO, 2011)
Se	0.20±0.01	0.22±0.02	0.40±0.10	0.055	0.055	0.40	0.06	0.40	0.03	0.15	0.06 (Lewis, 2019)
Fe	0.23±0.02	0.12±0.01	0.40±0.10	8	18	45	27	45	10	40	2.00 (FAO/WHO, 1984)
Mo	0.10±0.02	009±0.02	0.19±0.01	150	150	1100	50	2000	22	600	0.045 (Lewis, 2019)
Zn	0.08±0.01	0.07±0.01	0.13±0.02	11	8	40	11	40	5	12	3.00 (Lewis, 2019)
Co	0.07±0.01	0.02±0.00	0.08±0.02	ND	ND	ND	ND	ND	ND	ND	0.0014 (Kim et al., 2006)
Ni	0.06±0.01	0.04±0.01	0.10±0.02	ND	ND	1	D	1	ND	0.3	0.20 (FAO/WHO, 1984)
Mn	0.05±0.01	0.03±.01	0.07±0.01	2.30	1.80	11	2.60	11	1.50	3	3.00 (Lewis, 2019)
Cr	0.03±0.01	0.01±0.00	0.05±0.01	0.035*	0.025*	ND	0.030*	ND	0.015*	ND	0.002 (FAO/WHO, 1984)

Note. ND – not determined; DRIs – Dietary References Intakes; RDA – Recommended Dietary Allowances; AI – Adequate Intakes; *The value for Al is used when there are no calculated values for the RDA; UL – Tolerable Upper Intake Levels; FAO – Food Agriculture Organization of the United Nations; WHO – World Health Organization.

The concentration of chemical elements quantified in *C. adamantium* pulp samples is depicted in decreased order in Table 1.3. The average concentration of chemical elements in pulp collected on roadside followed in decreased order K > Pb > P > As >Fe > Se > Mo > Zn > Co > Ni > Mn > Cr; bush: K > Pb > P > As > Se > Fe > Mo > Zn > Ni > Mn > Co > Cr, and farm-margin: K > Pb > P > As > Se > Fe > Mo > Zn > Co > Ni > Mn > Cr. The concentrations of Pb, As and Cr in the present study are higher compared with the average reported for fruits and pulp collected in areas with a lower exposure to contaminants produced by anthropogenic activities (Vallilo et al., 2006; Lima et al., 2016; Lima et al., 2017), that exceed the FAO/WHO permissible limit recommended for edible berries and small fruits (FAO/WHO, 1984; Kim et al., 2006; WHO, 2012; FAO/WHO, 2011; Lewis, 2019). On the other hand, high concentrations of Mo, Co, Ni and Mn were reported with the occurrence of these chemical elements in natural environments (Nagajyoti et al., 2010; Bing et al., 2011).

In general, the average of all chemical elements quantified in *C. adamantium* pulp followed a decreasing order K > Pb > P > As > Se > Fe > Mo > Zn > Co > Ni > Mn > Cr. The one-way ANOVA test values considering the concentrations of each element were in collected in the three sites; then, we compared the pairs roadside/bush, roadside/farm-margin and bush/farm-margin. The concentrations of K, Pb, Se, Fe, Mo, Zn, Co, Ni and Mn in the pulp collected in roadside/bush points showed statistical differences ($p < 0.05$). However, significant differences ($p > 0.05$) were not observed when comparing the concentration of each chemical element found in *C. adamantium* pulp collected in roadside/bush/farm-margin.

Thus, it was observed that the concentration behavior of chemical elements decreased from the roadside (500 m) to bush (1000 m) and increased to farm-margin (3000 m). However, the concentrations of Pb and Se increased from the roadside to the bush and more toward the farm-margin, as illustrated in Figure 1.4.

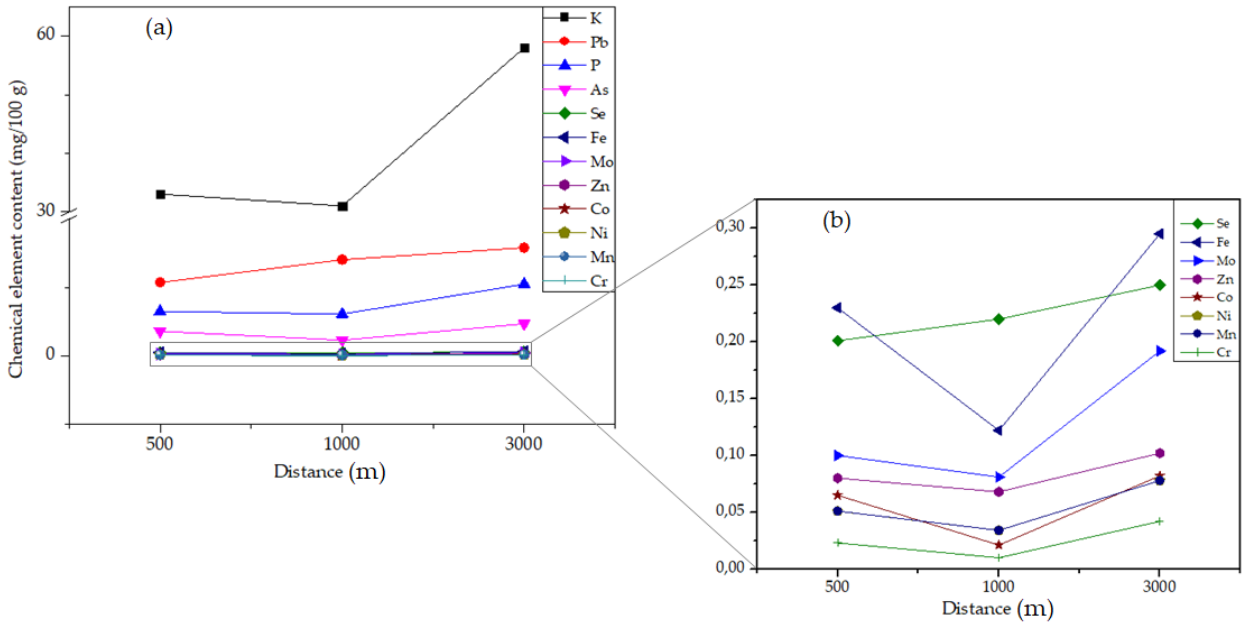

Figure 1.4. Behavior of the chemical elements' quantities distribution in *Campomanesia adamantium* pulp collected in three different sites: roadside (500 m); bush (1000 m); and farm-margin (3000 m), quantified by ICP OES (mg 100 g^{-1}): (**a**) chemical element content > 1 mg 100 g^{-1}; (**b**) chemical element content ≤ 0.4 mg 100 g^{-1}.

Table 1.3. list the levels of chemical elements quantified (mg 100 g^{-1} ± SD) in the *C. adamantium* pulp compared with the limit specification of RDAs/AI values for males and females (31–50 y), pregnant women (31–50 y) and children (4–8 y) (IMNA, 2006), and FAO/WHO and WHO (FAO/WHO, 1984; Kim et al., 2006; FAO/WHO, 2011; WHO, 2012; Lewis, 2019) permissible levels for fruits and food.

The percentages of chemical elements in the pulp were calculated from the mean values (Table 3) based on RDA, AI, UL, and FAO/WHO and WHO limits (FAO/WHO, 1984; Kim et al., 2006; FAO/WHO, 2011; WHO, 2012; Lewis, 2019), while the studied chemical elements were qualified based on the FDA parameters (10–19% for "good source" of nutrition, and ≥ 20% for "excellent source") (USDA, 2021).

Potassium (K) concentrations in roadside (33.02 ± 0.01 mg 100 g^{-1}), bush (31.02 ± 0.01 mg 100 g^{-1}) and farm-margin (58.01 ± 1.34 mg 100 g^{-1}) pulp correspond to proportions ≤ 1% for males, females, pregnant women, and children to 4700 mg day^{-1} by RDA parameters. The UL of K has no established values for males, females, pregnant women and children. The K content in this pulp was the lowest (3510 mg day^{-1}) FAO/WHO limit (WHO, 2012). According to FDA parameters, this pulp is not a good source of K (USDA, 2021). The K concentrations in this pulp are lower than 130 – 253 mg 100 g^{-1}, as reported in previous studies for *C. adamantium* fruits and pulp (Vallilo et al., 2006; Lima et al., 2016; Lima et al., 2017), which can be explained by the

higher levels of metalloids and heavy metals observed in this pulp which can reduce content, such as Cr, which results from intense anthropogenic activity (Houri et al., 2020). However, K concentrations in this pulp are near blueberry and alfalfa (39 mg 100 g^{-1}) (USDA, 2019). The health benefit of K in the body is associated with blood pressure regulation, stroke risk reduction, preventing renal system dysfunction, decreasing urinary calcium excretion, reducing kidney stone formation and osteoporosis disease (He and MacGregory, 2001), regulating blood lipid concentrations (Bing et al., 2011) and maintaining bone and cardiovascular health (Aburto et al., 2013; Weaver, 2013; Jeong et al., 2020).

Lead (Pb) concentrations in roadside (5.36 ± 0.02 mg 100 g^{-1}), bush (7.02 ± 0.01 mg 100 g^{-1}) and farm-margin (7.88 ± 1.05 mg 100 g^{-1}) pulp correspond to 26,800%, 35,100% and 39,480% by 0.02 mg day^{-1} FAO/WHO parameters (FAO/WHO, 2011). The RDA and UL parameters for Pb have no established values for adults and children. Although, according to FDA parameters, this pulp can be considered an excellent source of Pb (USDA, 2021), this chemical element (Pb) is hazardous to human health, as bellow described. In this pulp, Pb concentrations are lower than 0.06 mg 100 g^{-1}, as reported in previous studies for fruits of *C. adamantium* (Vallilo et al., 2006). On the other hand, Pb contents in this pulp are near those of the other edible fruit such as apple (2.35 mg 100 g^{-1}), mango (6.72 mg 100 g^{-1}) (Elbagermi et al., 2012) and tomato (5.41 – 11.73 mg 100 g^{-1}) (Nitu et al., 2019). The risk of consuming food with a high amount of Pb is correlated with intelligence reduction, bone joint weakness, accelerated bone maturation, increased blood pressure, spontaneous abortion, renal dysfunction, allergic diseases (Vasconcelos Neto et al., 2019), respiratory and cardiovascular diseases (Cweilag-Drabek, 2020).

Phosphorus (P) concentrations in roadside (3.24 ± 0.02 mg 100 g^{-1}), bush (3.04 ± 0.02 mg 100 g^{-1}) and farm-margin (5.24 ± 0.8 mg 100 g^{-1}) pulp correspond to proportions ≤ 1% for males, females and pregnant women (700 mg day^{-1}) and children (500 mg day^{-1}) by RDA parameters. The P contents correspond to values ≤ 0.2% for males/females (4000 mg day^{-1}), pregnant women (3500 mg day^{-1}) and children (3000 mg day^{-1}) by UL limits. The P concentrations of the roadside, bush and farm-margin pulp correspond to proportions < 1% to 700 mg day^{-1} by FAO/WHO limits (WHO, 2012; Lewis, 2019). Based on the FDA parameters, this pulp is not a good source of P (Lewis, 2019). Indeed, P concentrations in this pulp are lower than 17 – 196 mg 100 g^{-1} reported in previous studies on fruits and pulp of *C. adamantium* (Vallilo et al., 2006; Lima et al., 2016; Lima et al., 2017). However, P concentrations in this pulp are near of blackberry and watermelon (5 – 11 mg 100 g^{-1}) (USDA, 2019). The health benefit of P concentration is related to bone mineralization, cell energy generation, cardiovascular regulation and neuromuscular function

(Cooke, 2017), and the modulation of short-chain fatty acid gut bacteria producers (Trautvetter et al., 2018).

Arsenic (As) concentrations in roadside (1.96 ± 0.04 mg 100 g^{-1}), bush (1.14 ± 0.03 mg 100 g^{-1}) and farm-margin (2.84 ± 0.52 mg 100 g^{-1}) pulp correspond to 19,600%, 11,400% and 28,400% by 0.01 mg day^{-1} FAO/WHO limits (FAO/WHO, 2011). The RDA and UL parameters for As have no established values for adults and children. While, according to FDA limits, this pulp can be considered an excellent source of As (USDA, 2021), this chemical element (As) is hazardous to human health, as bellow reported. The As contents in this pulp are higher than 0.07 mg 100 g^{-1}, as reported in previous studies on *C. adamantium* fruits (Vallilo et al., 2006) and are near those of edible vegetables such as lettuce (2.73 mg 100 g^{-1}) (Oliveira et al., 2017), *Colocasia antiquorum* (0.6 – 12.5 mg 100 g^{-1}), gourd leaf (0.8 – 15.8 mg 100 g^{-1}) (Huq et al., 2006), fish, seafood and seafood products (0.16 – 0.56 mg 100 g^{-1} (Oberoi et al., 2014). The risk of the consumption of food with a high amount of As is associated with cancers (skin, lung and bladder) (Huq et al., 2006), respiratory disease, gastrointestinal disorder, liver malfunction, neurocardiovascular dysfunction, anemia disorder, leucopenia and thrombocytopenia effects, diabetes (Santra et al., 2013), cytotoxicity and genotoxicity effects (Shankar et al., 2014).

Selenium (Se) concentrations in roadside (0.20 ± 0.01 mg 100 g^{-1}), bush (0.22 ± 0.02 mg 100 g^{-1}) and farm-margin (0.40 ± 0.1 mg 100 g^{-1}) pulp correspond to values < 1% for males and females (0.055 mg day^{-1}), pregnant women (0.06 mg day^{-1}) and children (0.03 mg day^{-1}) by RDA parameters. The Se contents in the roadside, bush and farm-margin pulp correspond to proportions of < 1% for males and females (0.40 mg day^{-1}), pregnant women (0.06 mg day^{-1}) and children (0.15 mg day^{-1}) by UL limits. The Se concentrations in roadside, bush and farm-margin pulps correspond, respectively, to 333.33%, 366.67% and 500% by 0.06 mg day^{-1} FAO/WHO limits (Lewis, 2019). In agreement with FDA parameters, this pulp is an excellent source of Se (USDA, 2021). The Se concentrations in this pulp are lower than the amount of 0.88 mg 100 g^{-1} reported in previous studies on *C. adamantium* fruits (Vallilo et al., 2006), and higher than that reported in grapes, apricot, kiwi, litchi, macadamia and pistachio (0.0001 – 0.007 mg 100 g^{-1}) and near that of the cashew nut (0.02 mg 100 g^{-1}) (USDA, 2019). Other studies have recommended 0.018 – 0.15 mg day^{-1} of Se adult quantity intake for children (4–6 y), 0.023 mg day^{-1} for adolescent males 10–18 y and 0.021 – 0.4 mg day^{-1} for adult females (19–65 y), 0.027 – 0.4 mg day^{-1} for males and 0.0204 mg day^{-1} (FAO/WHO, 2001; NIH, 2021). Food consumption with adequate amount of Se is linked to synthesis of selenoproteins (selenocysteine, glutathione peroxidase) (Rayman, 2012; Rayman, 2020), which Se act as an anti-inflammatory, antioxidant preventing

and decreasing cardiovascular diseases (CVD), Alzheimer's disease, cancers (cancers (thyroid, colorectal, prostate, lung, bladder), antimutagenic, immune system regulation, thyroid gland function regulation, (Rayman, 2012; Rayman, 2020; Kieliszek et al., 2022), diabetes mellitus, cancers (Stranges et al., 2007), improving male fertility (Behne et al., 1996; Vézina, et al., 1996), human neuropathies (Hill et al., 2004), hepatic steatosis and others (Miyata et al., 2020). However, the Se supplement and non-supplement are recommended for food quantified with deficiency or excessive of Se amounts, respectively, because this trace element is associated with metabolic diseases above reported (Rayman, 2012; Rayman, 2020).

Iron (Fe) concentrations in roadside (0.23 ± 0.02 mg 100 g^{-1}), bush (0.12 ± 0.01 mg 100 g^{-1}) and farm-margin (0.40 ± 0.10 mg 100 g^{-1}) pulp correspond to values < 4% by RDA parameters for males (8 mg day^{-1}), females (18 mg day^{-1}), pregnant women (27 mg day^{-1}) and children (10 mg day^{-1}). The Fe contents in the roadside, bush and farm-margin pulp correspond to < 1 % by UL parameters for males, females and pregnant women (45 mg day^{-1}) and children (40 mg day^{-1}). The Fe concentrations in roadside, bush and farm-margin pulps correspond, respectively, to 15%, 6% and 20% by 2.00 mg day^{-1}FAO/WHO limits (FAO/WHO, 1984). In concordance with FDA parameters, this pulp is not a good source of Fe (USDA, 2019). The Fe concentrations in this pulp are lower than the amount of 1 – 2.6 mg 100 g^{-1} reported in previous studies on fruits and pulp of *C. adamantium* (Vallilo et al., 2006; Lima et al., 2016; Lima et al., 2017). However, the Fe content of this pulp is between that of apple, guava and pineapple (0.12 – 0.29 mg 100 g^{-1}) (USDA, 2019). The health benefits of food consumption with Fe are improving maximal oxygen respiration and exercise performance, hemoglobin synthesis, electron transport, anemia prevention, deoxyribonucleic acid synthesis, gut microbiota modulation, neurodevelopment, immunity, pregnancy development (Abbaspour et al., 2014; Pasricha et al., 2014; Georgieff et al., 2019).

Molybdenum (Mo) concentrations in roadside (0.10 ± 0.02 mg 100 g^{-1}), bush (0.09 ± 0.02 mg 100 g^{-1}) and margin-farm (0.19 ± 0.01 mg 100 g^{-1}) pulp correspond to proportions ≤ 1% by RDA parameters for males and females (150 mg day^{-1}), pregnant women (50 mg day^{-1}) and children (22 mg day^{-1}). The Mo contents in the roadside, bush and farm-margin pulp correspond to values ≤ 0.2% by UL parameters for males and females (1100 mg day^{-1}), pregnant women (2000 mg day^{-1}) and children (600 mg day^{-1}). The Mo concentrations in roadside, bush and farm-margin pulp correspond, respectively, to 222.22%, 177.78% and 422.22% by 0.045 mg day^{-1}FAO/WHO parameters (Lewis, 2019). In agreement with FDA parameters, this pulp is an excellent source of Mo (USDA, 2019). However, the Mo concentrations in this pulp are lower

than amount of 0.4 – 30 mg 100 g^{-1} reported in previous studies on fruits of *C. adamantium* (Lima et al., 2016; Lima et al., 2017). The Mo food consumption is recommended for infants (0.015–0.04 mg day^{-1}) (WHO, 2003). The health benefit of Mo is correlated with toxicity prevention by several metabolites, reduction in aerosol organs irritability, night blindness, neurological damage, aches and pain (Moss, 1995; Schwarz et al., 2009; Novotny, 2011). The Mo concentrations of this pulp are between those of pea seeds and tomato (0.10 – 0.20 mg 100 g^{-1}) (Tsongas et al., 1980).

Zinc (Zn) concentration in roadside (0.08 ± 0.01 mg 100 g^{-1}), bush (0.07 ± 0.01 mg) and farm-margin (0.13 ± 0.02 mg 100 g^{-1}) pulp correspond to values < 2% by RDA limits for males and pregnant women (11 mg day^{-1}), females (8 mg day^{-1}) and children (5 mg day^{-1}). The Zn contents in the roadside, bush and farm-margin pulp correspond to 1% by UL parameters for males, females, pregnant women (40 mg day^{-1}) and children (12 mg day^{-1}). The Zn concentrations in this pulp correspond to 2.67%, 2.27% and 3.4% by 3 mg day^{-1} FAO/WHO limits (Lewis, 2019). Based on FDA parameters, this pulp is not a good source of Zn (USDA, 2019). The Zn concentrations in this pulp are lower compared with the amount of 0.2–0.5 mg 100 g^{-1} reported in previous studies on fruits and pulp of *C. adamantium* (Vallilo et al., 2006; Lima et al., 2016; Lima et al., 2017). However, the Zn amounts are between those of apple, grapes and tomato (0.04–0.17 mg 100 g^{-1}) (USDA, 2019). The health benefit of the consumption of Zn food is associated with preventing or reducing oxidative stress, infections (malaria, pneumonia and diarrhea), cell ageing, atherosclerosis, neuropsychological diseases, autoimmune and degenerative diseases, Alzheimer's disease, inflammation cytokine storms, cancers, diabetes mellitus, obesity, depression, gastrointestinal and reproductive organ dysfunction, retina disease, and improving fetal and improving fetal and childhood skeletal growth and development (Chasapis et al., 2012; Roohani et al., 2013; Ugarte et al., 2013).

Cobalt (Co) concentrations in roadside (0.07 ± 0.01 mg 100 g^{-1}), bush (0.02 ± 0.00 mg 100 g^{-1}) and farm-margin (0.08 ± 0.02 mg 100 g^{-1}) pulp correspond to 5,000%, 1,428.57% and 5,714.29% by 0.0014 mg day^{-1} WHO limits (Kim et al., 2006). The RDA and UL parameters for Co have no established value for adults and children. Conforming to FDA parameters, this pulp is an excellent source of Co (USDA, 2019). The Co concentrations in this pulp are than 8 mg/100 g reported in previous studies on *C. adamantium* pulp (Lima et al., 2017). The Co concentrations are between strawberries, apple, grapes, mango, tomato and orange (0.03 – 0.08 mg 100 g^{-1}) (Elbagermi et al., 2012). The risk of consuming food with a high amount of Co is correlated with inflammation and hypersensitivity reactions (Czarnek et al., 2015), neurological, cardiovascular and endocrine deficiency (Leyssens et al., 2017).

Nickel (Ni) concentrations in roadside (0.06 ± 0.01 mg 100 g^{-1}), bush (0.04 ± 0.01 mg 100 g^{-1}) and farm-margin (0.1 ± 0.02 mg 100 g^{-1}) pulp correspond to 6%, 4% and 10% for males, females, pregnant women, and 20%, 13.33% and 33.33% for children, respectively, by 1 mg day^{-1} and 0.3 mg day^{-1} UL limits. The Ni concentrations of the roadside, bush and farm-margin correspond to 30%, 20% and 50% by 0.2 mg day^{-1} FAO/WHO limits (FAO/WHO, 1984), respectively. The RDA parameters for Ni has no established value for adults and children. According to FDA parameters, this pulp is an excellent source of Ni (USDA, 2019). The Ni concentrations in this pulp are lower than 4.2 mg 100 g^{-1} reported in previous studies on fruits of *C. adamantium* (Vallilo et al., 2006). The Ni concentrations are between those of paw-paw, mango, watermelon and banana fruits (0.023 – 0.089 mg 100 g^{-1}) (Onianwa et al., 2000). Some articles reported that the health benefit of Ni food consumption is correlated with gut microbiota balance and welfare (Zambelli et al., 2013). However, other studies correlated Ni with hazardous conditions for human health such as cardiovascular, kidney and lung dysfunctions and oxidative stress (Genchi et al., 2020).

Manganese (Mn) concentrations in roadside (0.15 ± 0.01 mg 100 g^{-1}), bush (0.03 ± 0.01 mg 100 g^{-1}) and farm-margin (0.07 ± 0.01 mg 100 g^{-1}) pulp correspond to proportions ≤ 4% for males (2.3 mg day^{-1}), females (1.8 mg day^{-1}), pregnant women (2.6 mg day^{-1}) and children (1.5 mg day^{-1}) by RDA parameters. The Mn correspond to proportions < 2.5% for males/females and pregnant women (11 mg day^{-1}), and children (3 mg day^{-1}) by UL limits. The Mn concentrations in pulps of roadside, bush and farm-margin correspond to 1.33%, 1.00% and 2.33%, respectively, by 3 mg day^{-1} FAO/WHO parameters (Lewis, 2019). Based on the FDA parameters, this pulp is not good source of Mn (USDA, 2019). The Mn concentrations in this pulp are lower than the amount of 0.09 – 0.21 mg 100 g^{-1} reported in previous studies on fruits of *C. adamantium* (Vallilo et al., 2006; Lima et al., 2016; Lima et al., 2017). However, the Mn contents are near those of paw-paw and wheat (0.08 – 1.0 mg 100 g^{-1}) (Marles, 2017). The health benefit of the consumption of Mn food is associated with gut microbiota balance, regulating oxygen species and anemia conditions between mother and fetus and neurodevelopment (Lee et al., 2018; Kupsco et al., 2019; Lopez and Skaar, 2018).

Chromium (Cr) concentrations in roadside pulp was 0.03 ± 0.01 mg 100 g^{-1}, which corresponds to 116.67%, 83.33%, 100% and 50% for males (0.35 mg day^{-1}), females (0.025 mg day^{-1}), pregnant women (0.03 mg day^{-1}) and children (0.015 mg day^{-1}) by AI parameters, respectively. The Cr content of 0.01 ± 0.00 mg 100 g^{-1} in bush pulp corresponds to 350%, 250%, 300%, and 150% for males, females, pregnant women and children, respectively, by AI limits. The Cr content in farm-margin pulp was 0.05 ± 0.01 mg 100 g^{-1}, which corresponds to 70%, 50%,

60%, and 30% for males, females, pregnant women and children, respectively, according to the AI standard. The Cr concentrations in the roadside, bush and farm-margin pulp correspond to 6.67%, 20% and 4%, respectively, by 0.002 mg day^{-1} FAO/WHO limits (FAO/WHO, 1984). The RDA and UL parameters for Cr have no established values for adults and children. Whilst, in conforming to FDA parameters, this pulp is a good source of Cr (USDA, 2019), a high amount of this chemical element (Cr) is correlated with hazardous to human health, as follow reported. However, the Cr concentrations in this pulp are lower than the amount of 0.1 – 1.14 mg 100 g^{-1} reported in previous studies on *C. adamantium* pulp (Lima et al., 2016; Lima et al., 2017). The Cr contents are near edible fruits such as strawberry and melon (0.3 mg 100 g^{-1}) (Cherfi et al., 2014). The Cr food intake is correlated with oxidative, inflammatory diseases improvement (Morvaridzadeh et al., 2022).

1.5.2. Health risk assessment

The carcinogenic risk (CR) was calculated for three chemical elements Pb, As and Cr in pulp obtained from fruits collected in roadside, bush and farm-margin areas (Table 1.4.). The values of As and Cr were farm-margin > roadside > bush, while the Pb values differed (farm-margin > bush > roadside). The total cancer risk (R) values for farm-margin, roadside and bush were 1.5×10^{-3}, 1.1×10^{-3} and 6.2×10^{-4}, respectively, which were higher compared with the acceptable parameters (10^{-6} to 10^{-4}), showing the importance of these values in terms of their carcinogenic risk for *C. adamantium* pulp consumers of 10 g kg^{-1} day^{-1} (US EPA, 1989). The total cancer risk is presented in decreased order As > Pb > Cr, demonstrating that As is the main pollutant chemical element that can be correlated with several cancer incidences among all heavy metals found in this pulp. Furthermore, the total cancer risk incidence can be higher for those who consume the recommended intake of 400 g day^{-1} (WHO, 2021) of pulp from farm-margin, roadside and bush (6.1×10^{-2}, 6.3×10^{-2} and 2.5×10^{-2}, respectively), in the region crossed by a road of high large vehicle traffic and intensive modern agriculture. However, the total cancer risks for the consumption of 1 g kg^{-1} day^{-1} of pulp from the roadside, bush and farm-margin were estimated to 1.1×10^{-4}, 6.3×10^{-5} and 1.5×10^{-4}, respectively, which are near of within acceptable parameters (US EPA, 1989).

Table 1.4. Carcinogenic risk (CR), chronic daily intake dose (CDI, mg kg^{-1} bw day^{-1}) and hazard quotient (HQ) of chemical elements based on 10 g of *Campomanesia adamantium* pulp collected at three different sites from the road: roadside (500 m), bush (1000 m) and farm-margin (3000 m).

Samples		K	Pb	P	As	Se	Fe	Mo	Zn	Co	Ni	Mn	Cr
Roadside	CR	-	1.6×10^{-5}	-	1.0×10^{-3}	-	-	-	-	-	-	-	5.3×10^{-6}
	CDI	1.2×10^{-2}	1.9×10^{-3}	1.1×10^{-3}	6.9×10^{-4}	7.0×10^{-4}	8.1×10^{-5}	3.5×10^{-5}	2.8×10^{-5}	2.5×10^{-4}	2.1×10^{-5}	1.8×10^{-5}	1.1×10^{-5}
	HQ	-	4.7×10^{-1}	-	2.3×10^{0}	-	1.2×10^{-4}	7.0×10^{-3}	9.4×10^{-5}	8.2×10^{-4}	1.1×10^{-3}	1.3×10^{-4}	3.5×10^{-3}
Bush	CR	-	2.1×10^{-5}	-	6.0×10^{-4}	-	-	-	-	-	-	-	1.8×10^{-6}
	CDI	1.1×10^{-2}	2.5×10^{-3}	1.1×10^{-3}	4.0×10^{-4}	7.7×10^{-5}	4.2×10^{-5}	3.2×10^{-5}	2.5×10^{-5}	7.0×10^{-6}	1.4×10^{-5}	1.1×10^{-5}	4.0×10^{-6}
	HQ	-	6.2×10^{-1}	-	1.3×10^{0}	-	6.0×10^{-5}	6.3×10^{-3}	8.2×10^{-5}	2.4×10^{-4}	7.1×10^{-4}	7.6×10^{-5}	1.2×10^{-3}
Farm-margin	CR	-	2.4×10^{-5}	-	1.5×10^{-3}	-	-	-	-	-	-	-	8.8×10^{-6}
	CDI	2.0×10^{-2}	02.8×10^{-3}	1.8×10^{-3}	1.0×10^{-3}	1.4×10^{-4}	1.4×10^{-4}	6.7×10^{-5}	4.6×10^{-5}	2.8×10^{-5}	3.5×10^{-5}	2.5×10^{-5}	1.8×10^{-5}
	HQ	-	6.9×10^{-1}	-	3.3×10^{-0}	-	2.0×10^{-4}	1.3×10^{-2}	1.5×10^{-4}	9.4×10^{-4}	1.8×10^{-3}	1.2×10^{-3}	5.9×10^{-3}

The non-carcinogenic risks for chemical elements are summarized in Table 1.4. The CDI values of the chemical elements in fruit pulp were presented in decreased order for three collection sites: K > Pb > P > As > Fe > Se > Mo > Zn > Co > Ni > Mn > Cr for roadside, K > Pb > P > As > Se > Fe >Mo > Zn > Co > Ni > M > Cr for bush, and K > Pb > As > Se = Fe > Mo > Zn > Ni > Co > Cr for farm-margin. The ordered concentrations of chemical elements are different for Fe and Se from roadside, while these are Se, Fe, Ni and Co for the farm-margin compared with bush areas. The major chemical elements in the pulp in decreased order are farm-margin > roadside > bush, which signifies that the farm and road have spread these chemical elements to pollute fruits. In contrast, Pb and Se are ordered from farm-margin > bush > roadside, which explains that the highest amount of these chemical elements have spread from the farm.

The hazard quotient (HQ) values of the chemical elements in roadside pulp estimated in decreased order are As > Pb > Mo > Cr > Ni > Co > Fe > Mn > Zn; in bush pulp: As > Pb > Mo > Cr > Ni > Co > Fe > Zn > Mn; and in farm-margin pulp: As > Pb > Mo > Cr > Ni > Co > Mn > Fe > Zn. The contents of Mn, Fe and Zn are irregularly distributed in farm-margin, roadside and bush areas. The majority of chemical elements were ordered as farm-margin > roadside and bush, which explains that the farm and road are sources of higher amounts of these chemical elements. In contrast, Pb is ordered as farm-margin > bush > roadside, meaning that this chemical element has spread in a higher amount from the farm. The majority of chemical elements presented HQ < 1, while the highest values of As in the farm-margin, roadside and bush were 3.33, 2.30 and 1.34, respectively. Therefore, with a consumption of 10 g kg^{-1} day^{-1} of pulp, As could be the main cause of several cancer types and other chronic diseases.

1.6. CONCLUSIONS

According to RDA and UL limits, the pulp of *C. adamantium* collected in areas located between the road subject to high large vehicle traffic and intensive modern agriculture farming presented the lowest concentration of K, P, Se, Fe, Mo, Zn, Ni, and Mn. However, based on FAO/WHO parameters, the highest concentrations are Pb, As, Se, Mo, Co and Ni, and the lowest are K, P, Fe, Zn and Mn. The Cr concentration is above FAO/WHO and AI limits. Values of Pb, As, Se, Co and Cr are not established by RDA and UL standards, including K, which are not established for the last parameter. This pulp is an excellent source of Pb, As, Se, Mo, Co, Ni and Cr, while it is not a good source of K, P, Fe, Zn and Mn, based on FDA parameters. It is notable that plants that grow and develop between intensive anthropogenic and severe activities are contaminated by heavy metals such as Pb, As, Mo, Co, Ni, Mn and Cr. Additionally, the concentrations of these heavy metals increase, while K, P, Fe, and Zn decrease, except Se. Therefore, the consumption of plants collected in these environments can be a hazard to human health. Therefore, toxicological studies may be necessary to guarantee the safe consumption of edible plants collected in areas under intensive severe anthropogenic activities.

Overall, the estimated carcinogenic risk and total cancer risk in this pulp are represented by As, Pb, and Cr, which are in higher concentrations in pulp collected in farm-margin, followed by the roadside and bush. The primary crucial heavy metal is As, presenting HQ > 1 (3.33, 2.30 and 1.34 in pulp collected in farm-margin, roadside and bush, respectively). However, quantities ≤ 1 g daily intake of pulp obtained in these areas can decrease the total cancer risk and are within accepted parameters and HQ < 1 for all chemical elements assessed in this pulp. This demonstrated that modern intensive agriculture farms and areas crossed by roads of large vehicle traffic are sources of pollutants that contaminate fruits and vegetables that grow in surrounding areas.

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CHAPTER II

Fatty acid and mineral profile, physicochemical, oxidative and thermal characteristics of crude sunflower oil extracted by a domestic machine

2.1. ABSTRACT

Vegetable oils' adequate consumption is associated with energy acquisition, nutritional benefits, health improvement, and metabolic disease regulation. This study evaluated fatty acids composition, physicochemical, thermal, oxidative, and optical properties, and quantified trace elements in the sunflower oil extracted by a domestic cold-press machine. The oil presented linoleic (54.00%) and oleic (37.29%) primary of the unsaturated fatty acids (91.67%), which atherogenic (0.05), thrombogenic (0.16), hypocholesterolemic/hypocholesterolemic (21.97), peroxide (16.16), saponification (141.80) and relative density indices (0.92) demonstrated to be suitable for human consumption and possible health promotion. In addition, trace elements concentrations by IC OES were ordered Zn > Fe > Al > Cu > Mn > Cr. Concentrations of Zn, Fe, Al, Cu and Mn were lower than FAO/WHO and DRI/AI limits, while Cr concentrations exceeded the FAO/WHO limits, which can be used as an indicator of the polluted ambiance. Possible sunflower oil quantities daily consumption were calculated considering non-carcinogenic risk ($CR < 10^{-4}$), and total non-carcinogenic hazard index ($HI < 1$). Based on trace elements determined in this study, the suitable quantity of sunflower oil consumption varies according to individuals aged 8, 18, and 30 years shall be deemed 0.61, 1.46, and 1.65 g kg^{-1}, respectively, attending $HI = 0.99$, and $CR < 10^{-4}$.

Keywords: Cold-press, vegetable oil quality, trace elements, non-carcinogenic indices, oil quantity consumption

CAPÍTULO II

Perfil de ácidos graxos e minerais, características físico-química, oxidativa e térmica do óleo de girassol bruto extraído por máquina doméstica

RESUMO

O consumo adequado de óleos vegetais está associado a aquisição de energia, benefícios nutricionais, melhoramento da saúde e regulação de doenças metabólicas. Este estudo avaliou a composição de ácidos graxos, propriedades físico-químicas, térmicas, oxidativa e óptica, e quantificou elementos traços (oligoelementos) no óleo de girassol extraído por uma máquina doméstica de prensagem a frio. O óleo apresentou ácidos graxos linoleico (54,00%) e oleico (37,29%) os principais ácidos graxos insaturados (91,67%), nas quais os índices aterogênico (0,05), trombogênico (0,16), hipocolesterolêmico/hipercolesterolêmico (21,97), peróxido (16,16), saponificação (141,80) e densidade relativa (0,92) demonstraram ser apropriados para o consumo e possível promoção da saúde humana. Em adição, a concentração dos elementos traços por ICP OES foram ordenados Zn > Fe > Al > Cu > Mn > Cr. As concentrações de Zn, Al, Cu e Mn são menores que os limites da FAO/OMS e DRI/AI, enquanto que as concentrações de Cr excederam os limites da FAO/OMS, na quais podem ser usados como um indicador da poluição ambiental. As possíveis quantidades do consumo diária do óleo de girassol foram calculadas considerando-se os índices: risco não carcinogênico (CR < 10^{-4}), e total de risco não carcinogênico (HI < 1). Com base em elementos traços determinados neste estudo, a quantidade adequada de consumo de óleo de girassol varia de acordo com a idade dos indivíduos de 8, 18 e 30 anos, sendo considerada 0,61; 1,46 e 1,65 g kg^{-1}, respectivamente, atendendo HI = 0,99 e CR < 10^{-4}.

Palavras-chaves: prensagem a frio, qualidade do óleo vegetal, elementos traços, índices não carcinogênicos, quantidade do óleo consumido.

2.2. INTRODUCTION

2.2.1. Sunflower (*Helianthus annuus* L.)

The genera *Helianthus* L. is circumscribed to Asteraceae Bercht. & J. Presl family composed by herbs (1–4.5 m height), is native to North America and Central America, composed approximately by 70 species (US, 2022), and commercially worldwide produced for nutritional and medicine benefits (Guo et al., 2017). For nutritional and culinary, its seeds and sprout are largely used as a snack, salad garnish, and edible oil production for cooking and frying, whilst in medicine are used as antimicrobial, anti-inflammatory, antihypertensive, wound-healing, regulating cardiovascular diseases (Guo et al., 2017).

Two species *Helianthus annuus* L. and *H. tuberosus* L. are more known by used to produce food for humans and livestock, whilst others are grown for ornamentation and the rest are considered weeds (US, 2022). Fig. 2.1. illustrates the principal morphology characteristics is composed by inflorescence in the central single disc and outer ray petals flowers (featuring yellow color and sterile) and deciduous pappus (easily detached and falling at maturity), an imbricate involucre (composed by bracts) and paleate receptacle (Schilling, 2001).

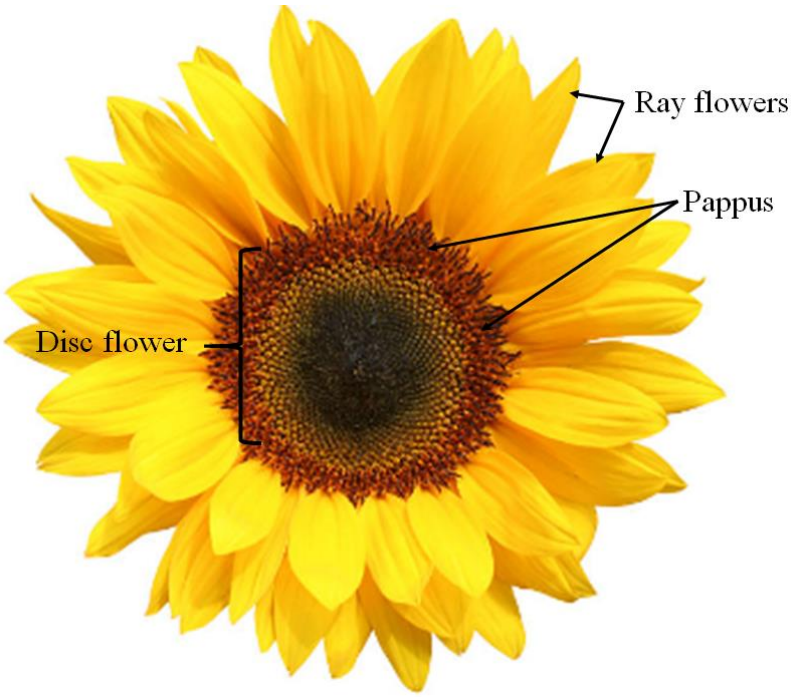

Figure 2.1. Principal morphology of the *Helianthus annuus* L.

2.2.2. Chemical compounds

Sunflower seed is a source of several substances such as unsaturated fatty acids represented by linoleic (61 – 66%) and oleic acids (22 – 27%) (Chowdhury et al., 2008), proteins, amino acids, polyphenols (flavonoids) (Pajak et al., 2014), phenolic acids (Weisz et al., 2009), vitamins A, B, C, K and E (Alagawany et al., 2015; Blicharska et al., 2014). Also were determined mineral elements as calcium (Ca), magnesium (Mg), manganese (Mn), chromium (Cr), arsenic (As), nickel (Ni), copper (Cu), zinc (Zn), iron (Fe) and others (Petraru et al., 2021). Others secondary substances are reported in seeds are terpenes, hydrocarbons, alcohol, alkaloids, saponins, steroids and others (Guo et al., 2017; Guo et al., 2019).

However, the most commercially available edible oils, including sunflower are extracted using petroleum solvents, with high toxicity levels for human health (Yara-Varón et al., 2017). Moreover, their essential components are removed during refining (chemical and physical) and bleaching processes, formatting short-chain compounds (esters, polymeric triacylglycerols, trans and free fatty acids, (hydro) peroxides, dienes and trienes, and others), which act as pro-oxidants (Gharby, 2022). These substances reduce vegetable oil shelf life and vital properties, which decrease healthy and nutraceutical properties, and associated with occurrence of several metabolic diseases in consumers (Siroma et al., 2022) due to the higher amount of free radicals increasing oxygen species (Carocho and Ferreira, 2013).

Moreover, its oil is potentially active against chronic diseases such as tumors, cholesterol, cancers (colon, digestive, breast and prostate), hypertension, hypercholesterolemia, type 2 diabetes mellitus, inflammatory, CVD and CHD (Adom and Liu, 2002; Liu, 2007; Nandha et al., 2014; Guo et al., 2017). These benefits are associated with synergic action of tocopherols, phytosterols, terpenoids, polyphenols, carotenoids, alkaloids, amino acids, proteins, vitamins, oleic acid, antioxidants and others anti-inflammatory substances (Kuo et al., 2022; Adeleke et al., 2020; Zeb et al., 2021). Due for reducing of the production of the reactive oxygen species (ROS) and reactive nitrogen species (RNS), consequently lowering disorder metabolites (superoxide ions ($^{\bullet}O_2^-$), hydroxyl (OH^-), hydrogen peroxide (H_2O_2), nitrate oxide (NO), etc.), which are linked with pro-inflammatory several metabolites: interleukins- (IL-1, -1β, -6, -8, -12), toll-like receptor 4 (TLR4), lipopolysaccharides (LPS, microbial component), hepatic nuclear factor-kB (NF-kB), tumor necrosis factor-alpha (TNF-α), inducible nitric oxide synthase (INOS), cyclooxygenase-2 (COX-2), lipoxygenase (LOX), cytochrome P450, nitric oxide (NO), G protein coupled receptor 120, and etc. potentially associated with oxidative stress and

metabolic diseases due high damage of cellular protein, lipids, DNA (Siroma et al., 2022; Zorgetto-Pinheiro et al., 2022; Rani et al., 2016).

Nevertheless, it is remarkable that food frying (pan and deep) using sunflower oil rich in linoleic fatty acid reduces its oxidation and thermal stability, which various hazardous substances to human health are forming (Ramadan, 2015). To reverse this scenario, blending cold pressed oils rich in natural antioxidants or their natural antioxidants (thymoquinone, a monoterpene) and tocopherols) from black cumin (*Nigella sativa*) with sunflower oil is high recommended to oils stability and for consumers health improving (Ramadan, 2015; Ramadan, 2013; Kiralan et al., 2017; Yildiz et al., 2021).

In addition, vegetable oils are source of macroelements as sodium (Na), potassium (K), calcium (Ca), magnesium (Mg), phosphorus (P) and microelements: iron (Fe), selenium (Se), manganese (Mn), chromium (Cr), zinc (Zn), aluminum (Al), barium (Ba), strontium (Sr), tin (Sn), copper (Cu), cobalt (Co), thallium (Ti), which are essential or toxic for human health when consumed in large quantities (Melo et al., 2019; Martinec et al., 2019). However, some chemical elements are concerning: arsenic (As), cadmium (Cd) nickel (Ni), lead (Pb), mercury (Hg) and Cu are found in edible vegetable oils, which are toxic (Astolfi et al., 2021; Mukhametov et al., 2020; Farzin et al., 2014) and carcinogenic for consumers even at a low amount (Tchounwon et al., 2012). On the other hand, the presence of Ca, Co, Mg, Fe, Zn, Cu, Mn, Sn and Ni accelerate vegetable oils oxidation affecting their flavor, freshness, storability and toxicity (Astolfi et al., 2021; Zhu et al., 2011). Therefore, cold pressing and filtration are the alternatives of the several methods to obtain healthy edible vegetable oils associated with good fatty acids and antioxidants composition, and lower amount of hazardous chemical elements (Rounizi et al., 2021; Fathollahy et al., 2021; Serra et al., 2019).

Interestingly, it is increasing the consumption of the unrefined edible vegetable oils, including sunflower oils obtained by cold pressed due to the high amount of natural antioxidants (tocopherols, phytosterols, carotenoids, etc.), waxes, whilst presenting low content of free fatty acids and phospholipids (Romanić et al., 2020; Mazaheri et al., 2019). In addition, some studies have reported nutritional qualities correlated with physicochemical properties, trace elements, fatty acids composition and essential components in sunflower oil obtained by cold pressing (Petraru et al., 2021). Therefore, a domestic cold press machine is mostly recommended to obtain healthy edible vegetable oils, due their higher amount of antioxidants presence and fatty acids composition and lower amount of heavy metals contaminants (Melo et al., 2019).

Although some studies have been carried out using different types of oils and extraction conditions, there is scarce information in the literature on the quality and mineral composition

of sunflower oil extracted by a domestic cold-pressing machine, as well as risk assessment for human health due to the ingestion of this type of oil containing metals.

Thus, the sunflower oil benefits and mineral amount concerning for human health justify in this work evaluating fatty acid composition, physicochemical and optical properties, thermal and oxidative stability of the sunflower oil samples cold extracted using a domestic machine. In addition, the chemical elements Mg, Cr, Mn, Fe, Co, Ni, Cu, Zn, Cd, Al, Pb, As and Se in sunflower oil were quantified using inductively coupled plasma optical emission spectroscopy (ICP OES) and the results were compared with DRI/AI* and FAO/WHO parameters.

2.3. OBJECTIVES

2.3.1. General objective

Evaluate of the fatty acids composition, physicochemical and optical proprieties, thermal and oxidative stability, and mineral composition of crude sunflower oil extracted using a domestic extraction machine.

2.3.2. Specific objectives

- Determinate the fatty acids profile of sunflower oil;
- Calculate fatty acids nutritional indices;
- Determinate the identity and quality characteristics of sunflower oil;
- Evaluate the oxidative stability of sunflower oil;
- Evaluate thermal stability of sunflower oil;
- Evaluate the optical proprieties of sunflower oil;
- Quantify mineral composition of sunflower oil;
- Compare mineral composition with Dietary reference intakes and adequate intake (DRI/AI*) and Food and Agriculture Organization of the United Nations and the World Health Organization (FAO/WHO) parameters;
- Calculate the risk of non-carcinogenic indices for human health.

2.4. MATERIAL AND METHODS

2.4.1. Sunflower seed, oil preparation, seed and oil moisture and lipid quantification

Sunflower seeds obtained in ten different farms in Campo Grande, Mato Grosso do Sul state, Brazil, in September 2020. Seeds mixed, immediately dried in an air circulation oven at 40 ^{o}C for 48 h. From dried hull seeds, using cold-pressed domestic machine extractor equipped with stainless continuous screw and drainage hole with internal filter to collect fresh oil (Yoda Nut & Seed Cold Press Oil Extractor-Gourmet Extractor, oil Natural, Homeup, (Yoda Europe, Cluj-Napoca, Romania) (Fig. 2.2.). Immediately, oil placed into amber and hermetic glass bottle, and then used for analysis.

Figure 2.2. Fresh oil extraction process. (**a**) Sunflower seeds; (**b**). Cold press machine oil extractor; (**c**). Container with crude sunflower oil.

Moisture content was measured using milled seeds (1.0 g) subjecting in the over at 105 ^{o}C for 60 min, then the sample rested in a desiccator, until achieving constant weight. For the filtered sunflower oil (1.4 g) relative humidity was determined by the Karl Fischer technique (KEM MKC-610 Karl Fischer Moisture Titrator, Japan).

Free fatty acids (FFAs) were extracted using Soxhlet extractor with petroleum ether as solvent at 60 ^{o}C for 6 h (Bligh and Dyer, 1959). Then, the solvent removed, and the extract subjected in the oven at 105 ^{o}C for 120 min, the sample cooled in desiccator until constant weight.

2.4.2. Methylation and fatty acids profile

Fatty acids methyl esters (FAMEs) of the sunflower oil were obtained at ambient temperature. Samples (157 mg) were weighed into assay tube, then saponified with methanolic NaOH 0.5 N (4 mL), esterified with a mixture of H_2SO_4 and NH_4Cl in methanol (5 mL), after hot bath added saturated solution of NaCl (4 mL), and finally added hexane (5 mL) (Maia and Rodrigues-Amaya, 1993).

FAMEs were analyzed using a gas chromatograph (model CP-3800, Varian, Santa Clara, CA, USA) equipped with flame ionization detector, a split/splitless injector, and stationary phase fused silica capillary column of polyethylene glycol (carbowax 20 M, length 30 m × 0.25 mm, Quadrex, Santa Clara, CA, USA). Operational parameters were followed for chromatography: the injector and detector temperatures were 250 ºC. The column temperature was programmed to 80 ºC for 2 min, followed by a ramp of 4 ºC/min up to 220 ºC and kept for 13 min; hydrogen carrier gas with 1 mL min^{-1} flow and injection volume 1 μL. Retention times were compared with the respective methyl ester standards (Supelco, F.A.M.E. mix C4:0 to C24:0, Sigma-Aldrich, Darmstadt, DA, Germany) (Dodds et al., 2005).

2.4.3. Fatty acids nutritional quality indices

Sunflower oil nutritional quality evaluated according to its fatty acids composition assessed by three following indices:

Atherogenicity index (AI): (Ulbricht and Southgate, 1991)

$$\mathrm{AI} = \frac{\mathrm{C12{:}\,0} + (4 \times \mathrm{C14{:}\,0}) + \mathrm{C16{:}\,0}}{\sum \mathrm{MUFA} + \sum \mathrm{PUFA}} \quad (2.1)$$

Thrombogenicity index (TI): (Ulbricht and Southgate, 1991)

$$\mathrm{TI} = \frac{\mathrm{C14{:}\,0} + \mathrm{C16{:}\,0} + \mathrm{C18{:}\,0})}{(0.5 \times \sum \mathrm{MUFA}) + (0.5 \times \sum \omega 6) + (3 \times \sum \omega 3)} \quad (2.2)$$

Fatty acids hipocholesterolemic/hypercholesterolemic (HH) ratio: (Santos-Silva et al., 2002)

$$\mathrm{HH} = \frac{\mathrm{C18{:}\,1\omega 9} + \mathrm{C18{:}\,2\omega 6} + \mathrm{C20{:}\,4\omega 6} + \mathrm{C18{:}\,3\omega 3} + \mathrm{C20{:}\,5\omega 3} + \mathrm{C22{:}\,5\omega 3} + \mathrm{C22{:}\,6\omega 3}}{\mathrm{C14{:}\,0} + \mathrm{C16{:}\,0}} \quad (2.3)$$

2.4.4. Identity and quality characteristics of sunflower oil

Sunflower oil characterization was conducted according American Oil Chemist's Society (2004), in triplicate, for qualification parameters: acid (Ca 5a-40) and peroxide indexes (Cd 8-53); identity parameters: relative density (Cc 10a-25), iodine-Wijis method (d 1-25), and saponification values (Cd 3-25).

2.4.5. Determination of oxidative stability

Sunflower oil oxidative stability was determined by the Rancimat method (873 Metrohm Co, Basel, Switzerland) by accelerated oxidation according to the European Union standardized standard EN 14112 (2003). The analyses were performed subjecting 3.0 g of oil at a constant temperature of 100 ^{o}C under an airflow rate of 10 L h^{-1} through the samples, and then into measuring vessel containing 50 mL ultrapure water Mill-Q in which the conductivity generated by volatile products during the oil decomposition was measured as a function of time.

2.4.6. Thermal analyses: Thermogravimetry Analysis (TGA)/Derivative Thermogravimetry (DTG), and Differential Scanning Calorimetry (DSC)

TGA/DTG curves were obtained using TGA Q50 (TA Instruments, New Castle, DE, USA). Samples of sunflower oil (~ 6 mg) were added into a platinum pan from 10 to 550 ^{o}C at a heated rate of 2 ^{o}C min^{-1} under inert nitrogen and synthetic oxygen atmosphere gases at a flow rate of 60 mL min^{-1}.

In addition, DSC curves were conducted at DSC-Q20 equipment with RCS90 coupled to a cooling system (TA Instruments). The DSC curves were obtained in a calorimeter model DSC Q20 coupled to an RCS90 refrigeration system (TA Instruments). Approximately 3 mg of sunflower oil using aluminum crucibles (Tzero standard) as support and reference, at a heating rate of 10 ^{o}C min^{-1}, cycle heating followed by cooling to temperatures between –80 ^{o}C to 25 ^{o}C, under an inert nitrogen atmosphere with a flow of 60 mL min^{-1}.

Curves were obtained from TGA/DTG and DSC data, which were generated by Universal Analyses 2000 software version 3.7A (TA Instruments).

2.4.7. Optical molecular analyses: UV–Visible absorption and fluorescence spectroscopy

Sunflower oil was diluted in HPLC grade hexane (spectroscopic grade 99.9%) at a concentration of 10 g L^{-1} and from stock solution, different dilutions were prepared for spectra reading at 1×10^{-3} g L^{-1} and 0.05 g L^{-1} UV-Visible absorption measurements were made using spectrophotometer (Lambda 265 UV/Vis, Perkin Elmer, Waltham, MA, USA), which the spectra were collected in the 200–800 nm range.

Excitation-emission matrix fluorescence spectra map were obtained using a spectrofluorometer (Cary Eclipse, Varian). The excitation-emission maps of fluorescence were obtained by exciting the samples in the wavelengths between 240 and 450 nm in 5 nm steps and collecting the emission from 250–750 nm in 1 nm steps. The excitation and emission slits were 5 nm and the sensitivity of the detector was 600 V.

For the UV-Vis absorption and fluorescence spectroscopy analyses, sunflower oil samples were placed into four-sided quartz optical cuvette cell with a 10 mm light optical path.

2.4.8. Extraction induced by emulsion breaking procedure and trace elements quantification

The emulsion breaking procedure was according to Carneiro et al., (2020) with some modifications as described. A vessel tube containing a mixture of 3.0 mL of sunflower oil, 3.0 mL of ethanol stirred for 20 s on a vertex shaker, immediately added 3.0 mL of ultrapure water (conductivity 18.2 MΩcm (Millipore), Biocel, Germany), plus 0.76 mL of Triton x-100, and 3.0 mL of HNO_3, then stirred for 20 s on a vortex shaker. Immediately, the emulsion was subjected to heated block at temperature of 90 ± 1 ^oC using for 20 min for phase separation. The cooled aqueous phase was transferred into a vessel tube by micropipette and ultra pure water was filled to complete a final volume of 6.0 mL. The blank solution was prepared followed the procedure of the samples, but without adding oil. Then, using a technique of Inductively Coupled Plasma-Optical Emission Spectrometer (ICP OES) with an Axial Plasma (iCAP 6000 Series, Thermo Scientific, Cambridge, UK), trace elements (Cr, Mn, Fe, Cu, Zn, and Al) quantified in sunflower oil.

Standard solutions were prepared diluting a standard multi-element stock solution (SpecSol, Quinlab, Jacarei, SP, Brazil) containing 1000 mg L^{-1} of each element. Five different concentrations were used to build calibration curves for the quantitative analysis of oil. The concentration for the elements was 0.01–5.0 mg L^{-1} range. The setup of ICP OES instrumental conditions for elemental analysis was according previous used by Machate et al., (2021). Table 2.1. summarizes the operational condition used the ICP OES apparatus analysis as wavelength, limit of detection (LOD), limit of quantification (LOQ), and coefficient of determination (R^2) in current study.

One blank and nine calibration curves were generated using the following concentration: 0.005, 0.01, 0.025, 0.05, 0.1, 0.25, 0.5, 1.0, and 2.0 mg Kg^{-1} of each element standard.

An addition/recovery test for the elements under study was carried out in a sunflower oil sample by spiking 0.5 mg L^{-1} of each analyte. The method had a recovery interval of 80 – 110% for the spike 0.5 mg L^{-1}, to the established limit proposed by Association of Official Analytical Chemists (AOAC, 2002).

Table 2.1. The inductively coupled plasma optical emission spectroscopy (ICP OES) operating conditions for analysis.

Elements	Wavelength (nm)	LOD (mg kg^{-1})	LOQ (mg kg^{-1})	Coefficient of determination (R^2)
Al	396.152	0.0114	0.0380	0.9995
As	193.696	0.0048	0.0159	0.9996
Cd	228.802	0.0007	0.0024	0.9994
Co	238.892	0.0016	0.0054	0.9995
Cr	425.435	0.0015	0.0049	0.9994
Cu	327.396	0.0014	0.0048	0.9995
Fe	259.940	0.0193	0.0644	0.9991
Mg	285.213	0.0675	0.2251	0.9984
Mn	257.610	0.0004	0.0012	0.9996
Ni	221.647	0.0023	0.0077	0.9994
Pb	220.353	0.0070	0.0233	0.9996
Se	196.026	0.0080	0.0267	0.9997
Zn	213.856	0.0018	0.0060	0.9991

2.4.9. Human health risk assessment

The concentration of the chemical elements in sunflower oil were compared with the FAO/WHO recommended intake standards and hazards quotient. The non-carcinogenic was calculated according to the equation adopted by Machate et al., 2021. Cancer risk is the probability of an individual developing any cancer type over lifetime due to a specific exposure to a hazardous mineral. The chronic daily intake dose (CDI) of carcinogenic elements (g kg^{-1} day^{-1}) and slope factor (SF) of Cr is 0.5 g kg^{-1} day^{-1}, according Equation (2.4):

$$\text{Cancer Risk} = \text{CDI} \times \text{SF} \quad (2.4)$$

Cancer risk is a sum of individual chemical element in different exposure pathways to develop cancer in a person, with is the total cancer risk (CR). According US UPA (1989)

acceptable values to cancer risk range 10^{-6} to 10^{-4}, while values > 10^{-4} are considered inadmissible.

The human health risk of trace element consumption dose was calculated on the chronic daily intake dose (CDI, mg kg^{-1} day^{-1}) for a chemical contaminant element in the sunflower oil intake quantity CDI calculated according Equation (2.5):

$$\mathrm{CDI}_{oil} = \frac{\mathrm{C} \times \mathrm{IR} \times \mathrm{EF} \times \mathrm{ED}}{\mathrm{BW} \times \mathrm{AT}} \quad (2.5)$$

Where CDI_{oil} – chronic daily oil intake dose; C – concentration of chemical content in sample (mg kg^{-1}); IR – ingestion rate g day^{-1}; EF – exposure frequency (365 days $year^{-1}$); ED – exposure duration; BW – body weight (kg), estimated 26, 62 and 70 kg for 8, 18 and 30 years old, respectively. AT – average time (ED ×365 days $year^{-1}$).

The risk to human health by the intake of trace element contaminated food was estimated using a hazard quotient (HQ), which is a ratio of CDI and chronic oral reference dose (RfD), determined by the following Equation (2.6):

$$\mathrm{HQ} = \frac{\mathrm{CDI}}{\mathrm{RfD}} \quad (2.6)$$

The RfD values for the risk were previously established by the Joint Food and Agriculture Organization/World Health Organization Expert Committee on Food Additive (JECFA WHO, 2003) and the United States Environmental Protection Agency (US EPA, 2022). The RfD (mg kg^{-1} day^{-1}) values are: Al = 1.0; Cr = 0.003; Cu = 0.04; Fe = 0.7; Mn = 0.14; and Zn = 0.3 (US EPA IRIS, 2022). As shown in Equation (6), hazard quotient toxic risk on each trace element and their sum, Equation (2.7) (total non-carcinogenic hazard index) HI < 1, safe food consumption, while HI < 1, health risk food consumption.

$$\mathrm{HI} = \mathrm{HQ}_{Al} + \mathrm{HQ}_{Cr} + \mathrm{HQ}_{Cu} + \mathrm{HQ}_{Fe} + \mathrm{HQ}_{Mn} + \mathrm{HQ}_{Zn} \quad (2.7)$$

2.4. RESULTS AND DISCUSSION

2.4.1. Oil preparation, sunflower seed, oil moisture and lipid quantity

Sunflower seed (1.0 g) yielded 260 mg g^{-1} (26%) of oil, lower compared with industrially obtained using petroleum solvents (36–50%) (Rauf et al., 2017). However, oil extracted by cold-press yielded high amount of beneficial nutritional components such as tocopherols, phytosterols, terpenoids, phenolic acids, sterols, carotenoids, antioxidants, chlorophylls, waxes (C36 – C48), oleic and linoleic acids, phospholipids, unsaponifiable matters, and others, in contrast, the hazardous components (trace metals) were extracted in lower amounts (Serra et al., 2019; Gotor et al., 2016; Carelli et al., 2002). The cold-pressed technique is widely recommended because provide oils with healthy components, including their by-products (oilcakes) (Petraru et al., 2021), which their adequate consumption are correlated with regulating metabolic diseases (Adeleke and Babalola, 2020).

The moisture determination in sunflower seeds (1.0 g) yielded 72.70 mg g^{-1} (7.27%) of dehydrated water, whereas dry matter was 927.24 mg g^{-1} (92.72%), better for fatty acid and essential components integrity, which are important for nutrition functionality (Huang et al., 2021). Sunflower oil moisture (1.4 g) was 998 μg, (0.07%) of evaporated water, demonstrating the importance of this oil in food system quality, due less susceptibility to stress oxidative and lipid oxidation by the action of the pro-oxidants substances (Yun and Surh, 2012). Using refined flour of the sunflower seed (1.0 g) yielded 493.82 mg g^{-1} (49.38%) of FFAs, demonstrating its relevance for nutritional value and a good source of vegetable oil (Petraru et al., 2021).

2.4.2. Fatty Acids Profile

Table 2.2. presents a comparison of the composition and quantity of fatty acids yielded in this study with others obtained by n-hexane solvent, massively used by industries to extract commercial edible vegetable oils (Nde and Foncha, 2020). Sunflower oil presented the following fatty acid profile in decreased order: linoleic (54.00%) > oleic (37.29%) > palmitic (4.13%) > stearic (3.17%) > behenic (0.55%) > araquidic (0.20%) > lignoceric (0.17%) > gondoic (0.13%) > docosadienoic = tricosilic (0.08%) > eicosapentanoic (0.06) > α-linolenic (0.05%) > palmitoleic = margaric (0.04%) > meristic (0.03%). In this study, most quantified fatty acids, linoleic and oleic were proportionally presented at 1.44:1, whereas those extracted by n-hexane as solvent presented 3.24:1 (Petraru et al., 2021).

Table 2.2. Fatty acids composition of sunflower oil extracted by cold-press and n-hexane as solvent.

Fatty acids	Mean ± Standard deviation (%)
Miristic (C14:0)	0.03 ± 0.00
Palmitic (C16:0)	4.13 ± 0.05
Palmitoleic (C16:1)	0.04 ± 0.00
Margaric (C17:0)	0.04 ± 0.00
Heptadecenoic (C17:1)	0.02 ± 0.00
Stearic (C18:0)	3.13 ± 0.08
Oleic (C18:1n9c)	37.29 ± 0.24
Linoleic (C18:2n6c)	54.00 ± 0.39
α-Linolenic (C18:3n3c)	0.05 ± 0.00
Araquidic (C20:0)	0.20 ± 0.00
Gondoic (C20:1)	0.13 ± 0.00
Eicosapentanoic (C20:5n3c)	0.06 ± 0.00
Behenic (C22:0)	0.53 ± 0.01
Docosadienoic (C22:2)	0.08 ± 0.00
Tricosilic (C23:0)	0.08 ± 0.00
Lignoceric (C24:0)	0.17 ± 0.01
Σ SFAs	8.33
Σ MUFAs	37.48
Σ PUFAs	54.19
Total FAs	100
Atherogenic index	0.05.
Thrombogenic index	0.16
Hypocholesterolemic/hypercholesterolemic	21.97

Σ SFAs: sum of saturated fatty acids, Σ MUFAs: sum of monounsaturated fatty acids, Σ PUFAs: sum of polyunsaturated fatty acids, FAs: fatty acids, ND: non detectable, defined as < 0.05%.

According to our results, the sunflower oil extracted using a domestic cold press machine yielded a higher amount of oleic acid and the highest hypocholesterolemic (HH) ratio (Table 2.2.) compared with one that applied n-hexane (oleic, 19.81%; linoleic, 64.35%; HH, 0.16 (Petraru et al., 2021), massively utilized by industry to extract edible vegetable oils.

Therefore, long-term diets of vegetable oils rich in linoleic acid and the lowest hypocholesterolemic index are associated with prevalence and incidence of several metabolic diseases (diabetes mellitus, coronary and inflammatory diseases, cancer, obesity, dysbiosis, and others (Kuo et al., 2022; Machate et al., 2020a; Thies et al., 2003).

Moreover, in the human body, linoleic acid (LA) is converted on arachidonic acid (AA) belong to n–6 PUFAs family, which this last is pro-inflammatory precursor of prostaglandin and leukotriene synthesis at the cyclooxygenase (COX-2), lipoxygenase (LOX), and cytokines (TNF-α, IL-1, IL-1β, IL-6, IL-8, IL-12, NF-kB, NO, LTs cytochrome P450, protein coupled receptor 120), which compete with n–3 PUFAs enzymes during the biosynthesis pathway of

long- (LC-PUFAs) and very-long-chain fatty acids (VLC-FAs) associated with anti-inflammatory effects (Siroma et al., 2022, Zorgetto-Pinheiro et al., 2022; Rani et al., 2016).

2.4.3. Fatty acid nutritional quality indexes and characteristics of sunflower oil

Table 2.2. depicts nutritional quality indexes: atherogenicity index (AI), thrombogenicity index (TI) and, hipocholesterolemic/hypercholesterolemic (HH) ratio calculated 0.05, 0.16, and 21.97, respectively. Sunflower oil obtained by the cold-press machine presents better values associated with regulating of several metabolic diseases for consumers than another extracted by petroleum solvent (Chen and Liu, 2020).

Table 2.3. presents physicochemical profiles revealing that this sunflower oil is suitable for human consumption, and its averages can be associated with oxidative stability, authenticity, quality and identity (Mengistie et al., 2018).

Table 2.3. Physicochemical characteristics of sunflower oil compared with Codex Alimentarius parameters.

Parameters	Sunflower oil	Maximum values
Acidity index (mg KOH g^{-1})	0.54 ± 0.0	4.0 (Codex Alimentarius, 1999) **(a)**
Peroxide index (mEq kg^{-1})	16.61 ± 0.2	≤ 20 (Codex Alimentarius, 1981) **(a)**
Iodine index (g I_2 100 g^{-1})	131.53 ± 0.1	118–141 (Codex Alimentarius, 2015) **(b)**
Saponification index (mg KOH g^{-1})	141.80 ± 1.92	186–198 (Codex Alimentarius, 2015) **(b)**
Relative density (20 ^{o}C)	0.9164 ± 0.1	0.922 –0.927 (Codex Alimentarius, 1999) **(c)**

(a) Parameter for cold pressed and virgin oils; **(b)** parameters for refined sunflower oils; **(c)** parameter for crude sunflower oil.

The iodine index (131.53) was found between the Codex Alimentarius parameters, which correspond to unsaturated fatty acids (91.67%). On the other hand, this oil characterized by the lowest acidity and peroxide indices, demonstrating its lipid stability against rancidity due to the lowest autoxidation products (ketones, aldehydes, hydroxyl alkenals and dienes) formation which became off-flavor and toxic food for consumers associated with pro-oxidants action (processing manner, oxygen, heat, light, and metals) (Siroma et al., 2022; Tao, 2018). In addition, this oil presented lower saponification index (141.80 mg KOH g^{-1} oil) compared with parameters and other obtained using petroleum solvent (188 and 189 mg KOH g^{-1} oil) (Ivanova et al., 2022),

represented by the long-chain fatty acids (palmitic, stearic, oleic and linoleic acids), which can be used to identify these oils.

Relative density is the relevant parameter correlated with edible vegetable oil absorption and mass transfer rates during cooling or melting, better to lower values than the parameters (Muñoz et al., 2022). Sunflower oil obtained using a domestic machine presented a lower average (0.91) compared with extracted using solvents (0.92–0.96) (Mabaleha et al., 2007; Segatin et al., 2020). Although, these values represent small difference among them, however, other parameters above referred can be used to identify the authenticity and origin of these oils.

2.4.4. Determination of oxidative stability

Rancimat data revealed that the crude sunflower oil (91.67%) presented an induction period (IP) of 5.06 h (Fig. 2.3). This behavior can be attributed to natural antioxidants in this oil as well observed to refined sunflower oil (unsaturated 88.40%), which the IP shifted from 5.5 h to 7.5 h, respectively in control and sunflower oil added polyphenol, subjected under a temperature of 110 ºC with air flow 20 L h^{-1} (Gharby et al., 2014). Furthermore, unsaturation amount of fatty acids is another relevant characteristic that is inversely proportional to oxidative stability. Sunflower hybrid oils (H) which presented higher iodine value (IV) showed lower IP, for instance, e.g. H19: (IV = 127, IP = 3.32 h) has lower IP compared with H21 (IV = 81, IP = 9.55 h) (Lužaić et al. 2022). Moreover, among unsaturation fatty acids, cold pressed sunflower oil (SO) that presented higher amount of oleic than linoleic fatty acids were more oxidative stably H20 (57), as well as SO1 (oleic, 86.52% and linoleic, 5.49%, IP = 19.87 h), while SO2 (oleic, 18.52% and linoleic, 66.02%, IP = 6.42 h) (Symoniuk et al., 2018).

Therefore, to increase the stability of sunflower oil (rich in linoleic acid) is recommend to make blend with oil rich of stable antioxidants as tocopherols and thymoquinone or applying their natural antioxidants (Ramadan, 2013; Kiralan et al., 2017).

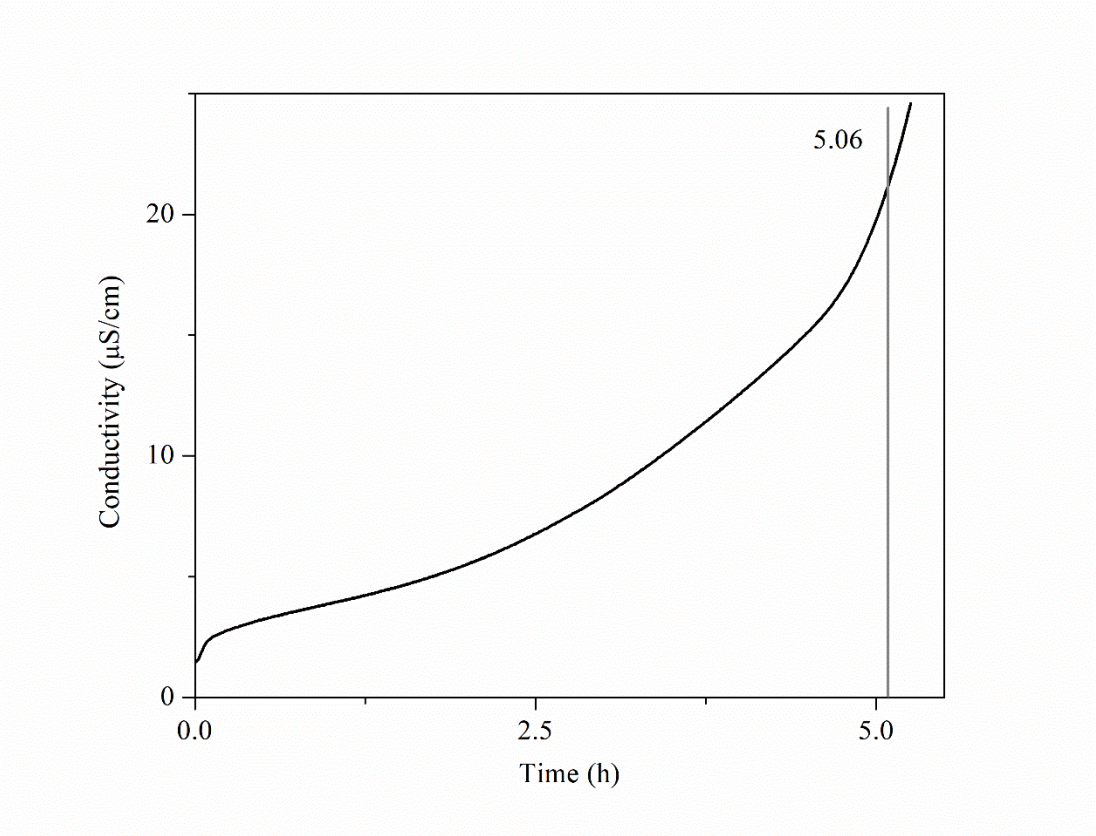

Figure 2.3. Conductivity versus time determined by the Rancimat method. Oxidation stability of sunflower oil conducted at 110 ºC with an airflow of 10 L h^{-1}.

2.4.5. Thermogravimetry Analysis (TGA)/Derivative Thermogravimetry (DTG), and Differential Scanning Calorimetry (DSC)

TGA/DTG curves of crude sunflower oil in the presence of synthetic air and nitrogen atmospheres are shown in Figs 2.4. and 2.5. The TGA/DTG curves of sunflower submitted under air synthetic atmosphere three steps illustrated in Figs. 2.4. and 2.5., and summarized in Table 2.4. The first step can be attributed to moisture linked by natural antioxidants (polyphenols, carotenoids, stanols, vitamins A and C), dimers, trimmers, polymers PUFAs, and others compounds formed from PUFAs (54.19%), represented by linoleic acid (54.00%). Also can be influenced by Zn (6.6228 mg kg^{-1}) and Fe (1.6637 mg kg^{-1}), metals with potential high oxidation level (Astolfi et al., 2021; Zhu et al., 2011). The first stage of thermal stability is the most important, as it demonstrated that this cold-pressed sunflower oil can be heated up to 125 °C without undergoing oxidative degradation.

The second step of mass loss is attributed to products formed in the first step plus MUFAs (37.48%) decomposition represented by oleic fatty acid (37.29%), whose double bonds of MUFAs are broken and become SFAs of the oil. The third step of mass decomposition is

attributed to above fatty acids plus SFAs (7.26%) (palmitic (4.13%) and stearic acids (3.13%) and waxes (C36 – C48), which the majority are represented by C36, C37, C40 and C41, previous reported in cold-pressed sunflower (Carelli et al., 2002). The minor last mass loss attributed to carbonaceous residue substances. This behavior was reported to commercial sunflower oil, although with more thermal stability compared with current study (Santos et al., 2002; Dweck and Sampaio, 2004; Correia et al., 2012). Moreover, cold press and unrefined oil present more oleic than refined one (Carocho and Ferreira, 2013), natural antioxidants (Zhang et al., 2020) and minerals, which are healthy and regulator of several metabolic diseases (Siroma et al., 2022; Correia et al., 2012; Petkova and Antova, 2019), economic and ecological benefits from food by-products (Lourenço et al., 2019) than refined ones. On other hand, synthetic antioxidants, butylated hydroxyanisole (BHA), butylated hydroxytoluene (BHT), tertbutyl hydroquinone (TBHQ), and others (Carocho and Ferreira, 2013; Sahin et al., 2020) replacing natural antioxidants in industrial edible vegetable oils, which are associated with prevalence of metabolic diseases (cellular damage, cancers) and environmental contamination (Wang et al., 2021; Lobo et al., 2010).

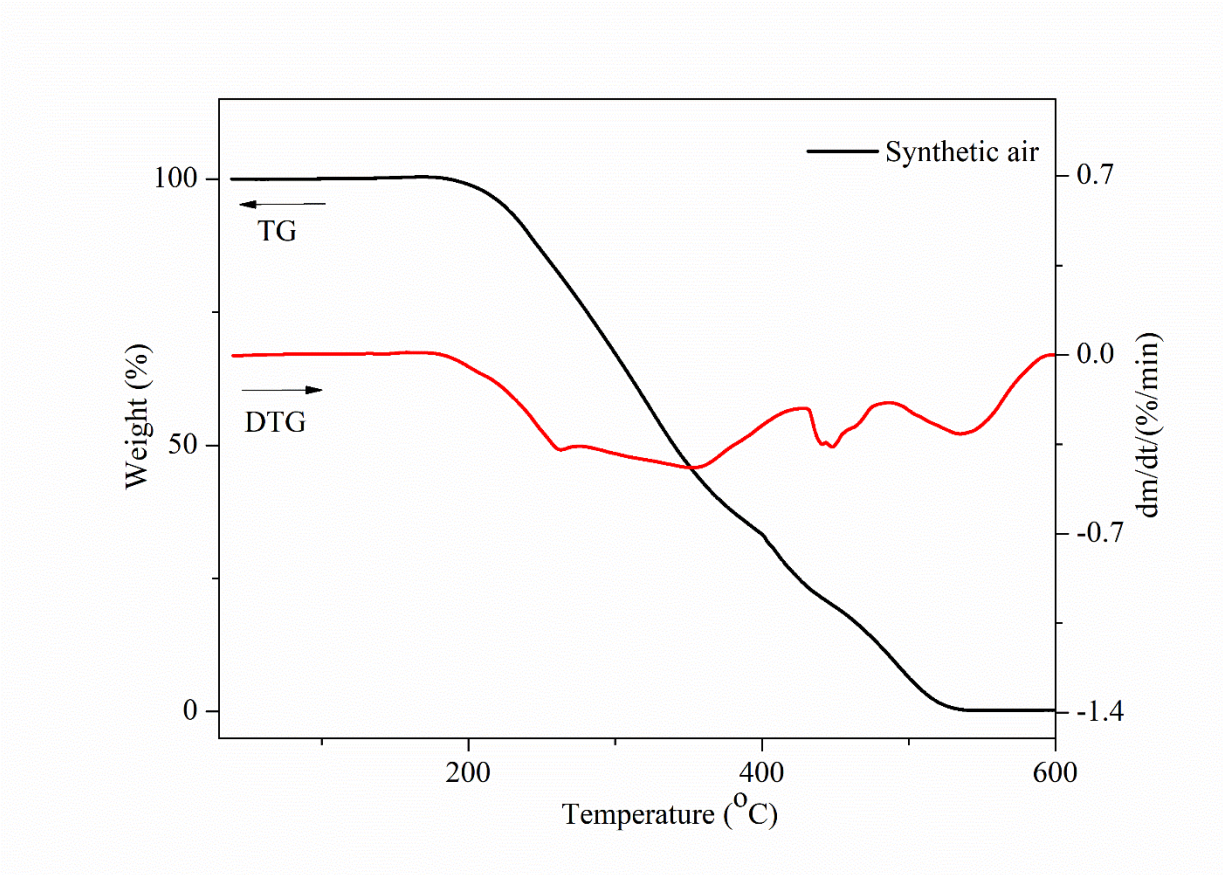

Figure 2.4. Thermal analysis of the sunflower oil. TGA/DTG curves of mass loss at 2 °C min^{-1} heating from 10–550 °C under synthetic air atmospheres flow at 60 mL min^{-1} in dynamic conditions.

In the nitrogen atmosphere, the oil showed one high step mass decomposition justified by a higher amount of unsaturated fatty acids (oleic and linoleic fatty acids) compared with SFAs. The minor last mass loss is attributed to formed carbonaceous residual substances.

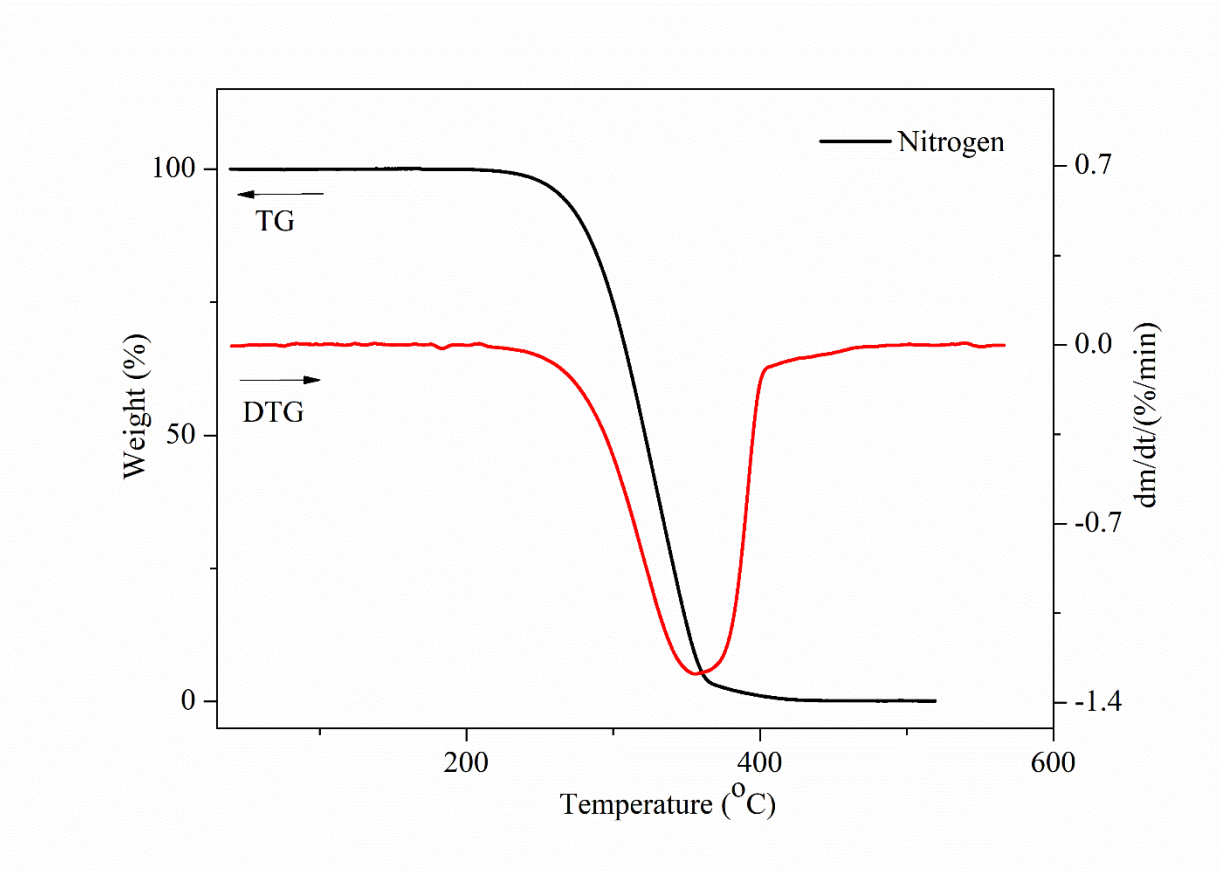

Figure 2.5. Thermal analysis of the sunflower oil. TGA/DTG curves of mass loss at 2 °C min^{-1} heating from 10–550 °C under nitrogen atmospheres flow at 60 mL min^{-1} in dynamic conditions.

Table 2.4. TGA/DTG curves of sunflower oil obtained under nitrogen and synthetic air atmosphere.

Sample	Atmosphere	Steps	Temperature range (°C)		Event mass loss (%)	Residual mass (%)
			Initial	Final		
Sunflower oil	Synthetic air	1°	126.16	254.34	15.48	0.21
		2°	254.34	392.17	49.74	
		3°	254.34	447.18	14.57	
	N_2	1°	236.19	465.90	99.09	0.33

DSC curves, Fig. 2.6. shows two exothermic crystallization peaks, which the first attributed to SFAs and MUFAs, with T_{onset} at –16.57 °C and enthalpy peak at 1.979 J/g, followed by a second peak representing PUFAs observed at –34.52 °C, with enthalpy peak at 1.466 J/g.

On the other hand, heating the sunflower oil, two peaks were also observed, the first peak corresponding to MUFAs, whose T_{onset} observed at –36.27 ºC and enthalpy at 1.866 J/g. The T_{onset} of the second peak appeared at –28.07 ºC and the enthalpy peak at 24.59 J/g.

In the current study, DSC results observed in lower temperatures than commercial sunflower oil due their fatty acids and natural antioxidants composition, which influence its thermal oxidative levels (Calligaris et al., 2007). The TGA and DSC analyses are used to qualify, authenticate, and recognize vegetable oils, thus avoiding their adulteration, falsification, and consumption of improper products.

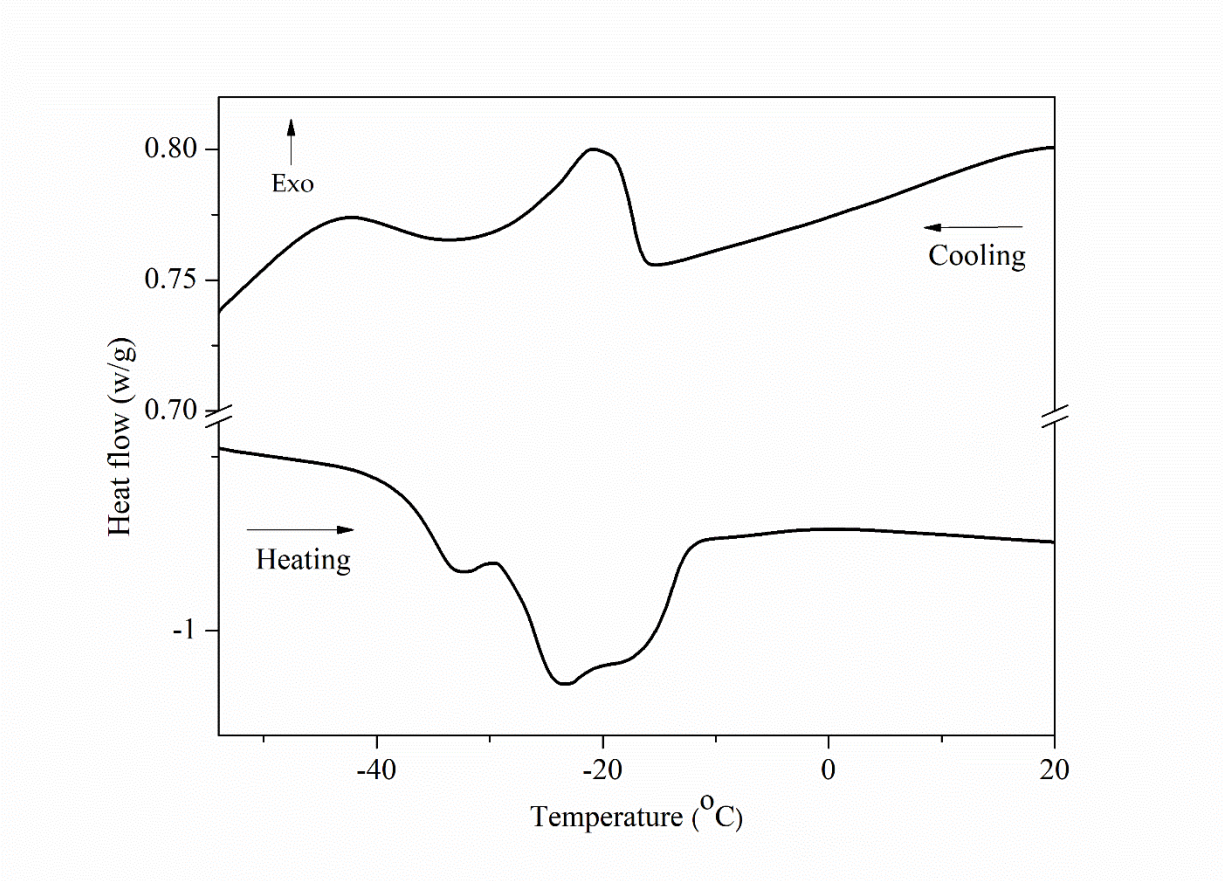

Figure 2.6. Thermal analysis of the sunflower oil curve of heating under N_2 atmosphere.

2.4.6. Optical molecular analyses: UV–Visible absorption and fluorescence spectroscopy

Fig. 2.7. illustrates sunflower oil UV-Visible absorption spectrum with two major absorbance regions one in the 223 – 236 nm range and other from 257 – 452 nm (inset of Fig. 2.7.). The first absorption band can be attributed to phytocholesterols (phytostanols), phytosterols and tocopherols (vitamin E). The region corresponding to carotenoids, and fatty acids (linoleic, oleic acids) (Gonçalves et al. 2014; Machate et al., 2020a). The consumption of

oils rich phytocholesterols, carotenoids, tocopherols and phytosterols, polyphenols, and unsaturated fatty acids are widely correlated with avoiding and regulating of several metabolic diseases (Machate et al., 2020b, Siroma et al., 2022).

The UV-Visible is used to monitor oxidation, identity and authenticity, as the absorption in the 400 – 520 nm range appears higher in adulterated sunflower oil (Popa et al., 2020).

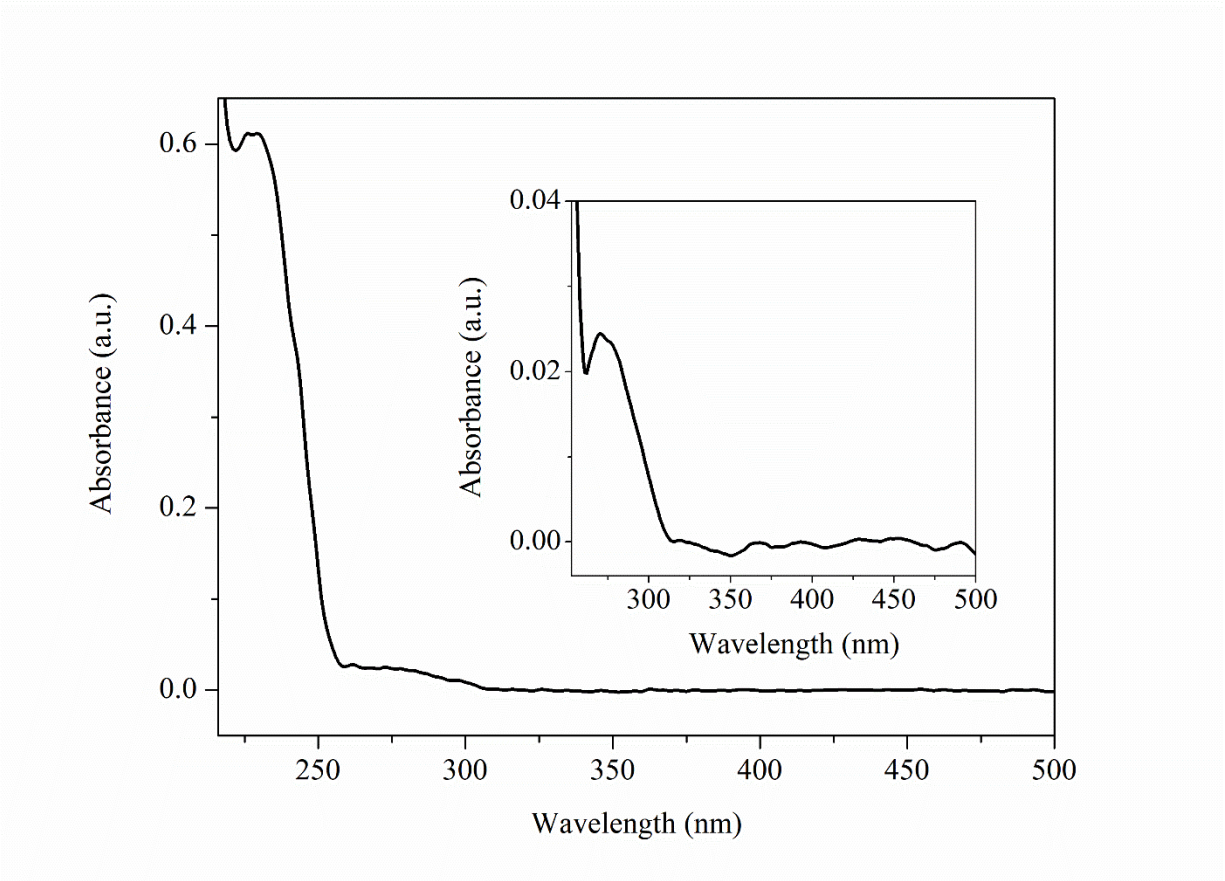

Figure 2.7. Optical molecular analysis used sunflower oil diluted in hexane HPLC 99.9% at 1×10^{-3} g L^{-1}. UV-Visible absorption spectrum wavelength collected between 200 – 800 nm.

Fig. 2.8. shows excitation–emission fluorescence map of sunflower oil exhibited two intense bands. The first band presents emission in the 297 – 327 nm range when excited between 290 nm and 310 nm. The second region of emission is observed between 360 and 445 nm due to an excitation in the 360 – 400 nm range. These fluorescence bands can be correlated with the presence of vitamin E (tocopherols, tocotrienols), carotenoids, chlorophyll, and unsaturated fatty acids in sunflower oil (Wu et al., 2014).

The fluorescence emission wavelength ranging from 400 to 500 nm is used to identify, qualify, authenticate original and adulterated vegetable edible oils due to the oxidation of fatty acids products and tocopherols (Li et al., 2015).

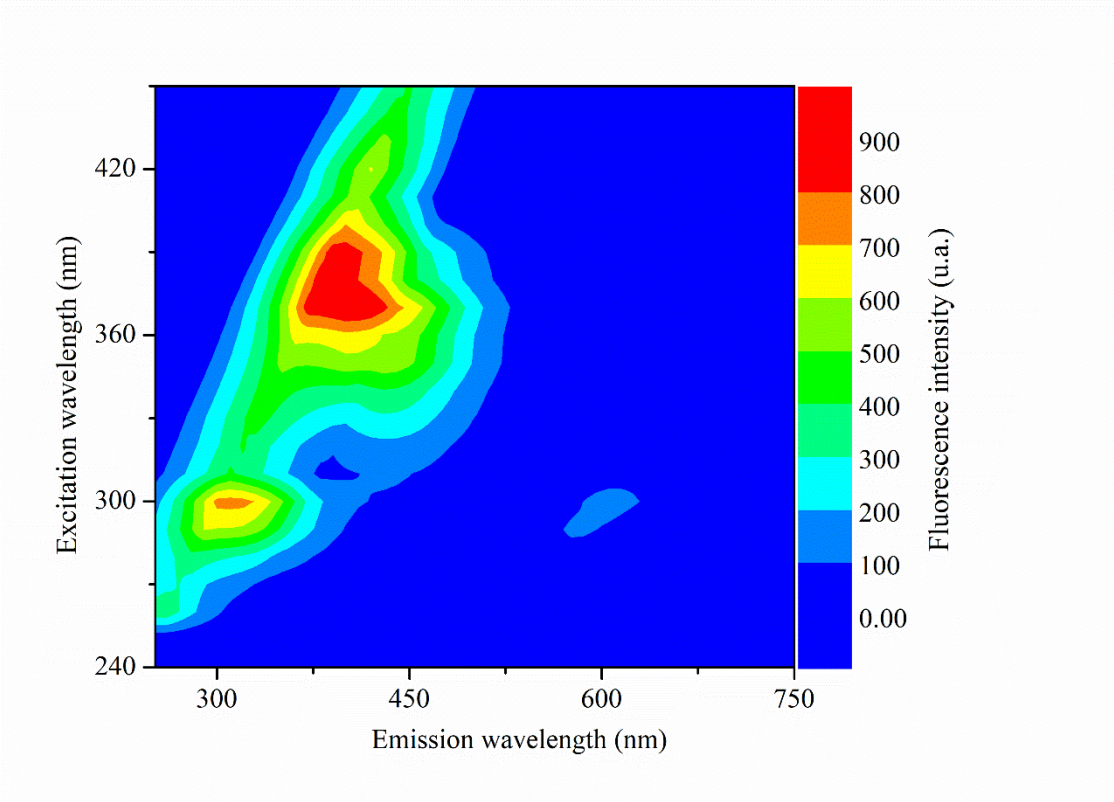

Figure 2.8. Emission-excitation fluorescence spectrum map with excitation obtained between 240–450 nm and emission (250–750 nm).

2.4.7. Trace elements concentration in sunflower oil

Table 2.5 summarize data of six trace elements quantified in sunflower oil compared to Codex Alimentarius contents for oils and dietary reference intakes (DRIs).

Beyond the endogenous biological processes, plants acquire minerals from the soil, besides exogenous processes (environment pollution by industries, transports, mechanization, fertilizers and pesticides used in agriculture), which influence raw material for edible plant production (Machate et al., 2021). On the other hand, minerals can contaminate edible vegetable oils during production, refining and storing processes.

Minerals (Ag, As, Be, Ca, Cu, Zn, Fe, Mg, Mn, Cd, Co, Na, K, Ni, Pb, V) are used to monitor and qualify edible vegetable oils: sunflower, olive (*Olea europaea*), hazelnut (*Corylus avellana*), and corn (*Zea mays*) in Turkey (Mendil et al., 2009), piqui (*Caryocar brasilense*), primrose (*Oenothera biennis*), avocado (*Persea americana*), coconut (*Cocos nucifera*), grape seed (*Vitis vinifera*), babassu (*Attalea speciosa*) and licuri (*Syagrus coronata*) in Brazil (Carneiro et al., 2020), and several varieties in China (Zhu et al., 2011) and Iran (Farzin and Moassesi, 2014).

Table 2.5. Trace elements in sunflower oil quantified by ICP OES (mg kg^{-1} ± SD) compared with nutritional recommendations for adult, pregnancy, lactation and children by DRIs: RDA/AI*, and FAO/WHO.

Trace elements	**Concentration (mg kg^{-1})**	**FAO/WHO (mg kg^{-1})**	**Dietary reference intakes (DRIs) and adequate intake (*AI) (mg day^{-1}) (2016)**								
			Children	Males		Females		Pregnancy		Lactation	
			8 y. old	18 y. old	30 y. old	18 y. old	30 y. old	18 y. old	30 y. old	18 y. old	30 y. old
Al	1.0401 ± 0.0199	5.00 (WHO, 2010)	ND	ND	ND	ND	ND	ND	ND	ND	ND
Cr	0.0242 ± 0.0049	0.002 (FAO/WHO, 1984)	0.015*	0.035*	0.035*	0.024*	0.025*	0.029*	0.030*	0.044*	0.045*
Cu	0.2791 ± 0.0094	0.90 (Lewis, 2019)	0.44	0.89	0.9	0.89	0.9	1	1	1.3	1.3
Fe	1.6637 ± 0.0393	14.00 (Lewis, 2019)	10	11	8	15	18	27	27	10	9
Mn,	0.2372 ± 0.0051	3.00 (Lewis, 2019)	1.5*	2.2*	2.3*	1.6*	1.8	2.0*	2.0*	2.6*	2.6*
Zn	6.6228 ± 0.0788	15.00 (Lewis, 2019)	5	11	11	9	8	12	11	13	12

Note. Elements As, Cd, Co, Mg, Ni, Pb, and Se < LOD; ND—not determined; DRIs – Dietary References Intakes; RDA – Recommended Dietary Allowances; AI – Adequate Intakes; *The value for Al is used when there are no calculated values for the RDA; FAO – Food Agriculture Organization of the United Nations; WHO – World Health Organization.

Mineral concentrations can be used as a relevant fingerprint to distinguish original from adulterated edible vegetable oils, promoting health benefit. The average concentrations of trace elements quantified in sunflower oil in decreased order are of Zn (6.6228 mg kg^{-1}) > Fe (1.6637 mg kg^{-1}) > Al (1.0401 mg kg^{-1}) > Cu (0.2791 mg kg^{-1}) > Mn (0.2372 mg kg^{-1}) > Cr (0.0242 mg kg^{-1}). Only Cr concentrations exceed 1210% than FAO/WHO limits, which can be the consequence of the vehicle fumes, fertilizers and pesticides used, as reported to the intensive agriculture modern farming, as well as the cultivated areas located near the road with high vehicle traffic (Machate et al., 2021; Rosa et al., 2022). In contrast, Zn, Fe, Al, Cu and Mn concentrations were lower than FAO/WHO and DRI/AI* limits. On the other hand, Cr and Zn concentrations were within between DRIs/AI* limits, while Cu, Fe and Mn concentrations were quantified lower than DRIs/AI* limits (Table 2.5.).

Furthermore, Al concentration was higher than quantified in industrial refined oils of avocado, primrose, babassu, licuri, pequi, grape seed and olive (0.04 – 0.52 mg kg^{-1}) (Carneiro et al., 2020). In contrast, Zn, Cu and Mn amounts were reported between those found in commercially refined oils of sunflower, olive, canola (*Brassica napus*), soybean (*Glycine max*), corn and hazelnut corresponding to 1.03–9.54, 0.05–4.504 and 0.04–1.76 mg kg^{-1}, respectively. The concentrations of Cr and Fe were lower than reported in sunflower, olive, canola, soybean, corn and hazelnut oils corresponding to 0.0126–7.106, 7.78–28.93 mg kg^{-1}, respectively (Mendil et al., 2009; Farzin and Moassesi, 2014; Bakircioglu et al., 2014).

Thus, in light of the acceptable concentrations of trace elements regarding to referential parameters, the consumption of the sunflower oil herein studied can be beneficial, because Cr, Cu, Fe, Mn and Zn play essential physiological roles in defense response, protein construction, enzymatic reactions, signaling pathways, regulation of oxidative stress and metabolic diseases, and others (Goldhaber, 2003). However, lower Al concentrations is associated with a reduced occurrence of metabolic diseases and dysfunctions, such as cancers, Alzheimer's and Parkinson's diseases, inhibit enzymatic cytotoxic and neurotoxic reactions, gut imbalance, skeletal disorders, and others (Machate et al., 2021).

2.4.8. Health risk assessment

Most studies reported daily consumption of vegetable oils from 25–30 g kg^{-1} (Zhu et al., 2011; Rong et al., 2021) and others were calculated approximately 312 g kg^{-1} (unsaturated, 278 g kg^{-1}; saturated, 31 g kg^{-1}; and trans fatty acids 3 g kg^{-1}) for the total energy intake per day (WHO, 2020). Others studies demonstrated that quantities of cooking oils depend on type

of dishes: pure vegetables range from 9–167 g kg^{-1}, with mean of 56 g kg^{-1}, pure meat from 4–353 g kg^{-1} (142 g kg^{-1}) and mixed meat-vegetable 7–394 g kg^{-1} (110 g kg^{-1}) (Pu et al., 2019).

However, the calculated quantities of the sunflower oil daily intake (g kg^{-1}) in comparison with other studies based on concentration of trace elements regarding to acceptable non-carcinogenic risk (CR) $<10^{-6}$ to 10^{-4} and total non-carcinogenic hazard index (HI) < 1 are summarized in Table 2.6. Based on country origin, it is remarkable that the quantity of the sunflower oil daily intake is independent of the obtained by cold-press (Brazil and Romania) or petroleum solvent extraction (China and Turkey). Regarding to the trace elements concentrations in sunflower oil, the quantity of sunflower oil daily intake by individuals aged 8, 18, 30 years old are respectively describe in decreased order for Brazil (0.61, 1.46, 1.65 g day^{-1}) > China (0.41, 0.99, 1.12 g day^{-1}) > Romania (0.037, 0.08, 0.099 g day^{-1}) > Turkey (0.0097, 0.0234, 0.0264 g kg^{-1}) > Turkey (0.0093, 0.0224, 0.0253 g kg^{-1}) (Table 2.6.).

Thus, given the findings of the current study (mineral concentrations), it seems relevant to explore the calculating amount of vegetable oil consumption per day based on trace elements quantified from different origins to be used in health promotion and regulation of several metabolic diseases.

Table 2.6. Non-carcinogenic risk (CR), hazard quotient (HQ), and total non-carcinogenic hazard index (HI) of trace elements on ingestion rate (IR g kg^{-1}) of sunflower oil obtained by cold-pressed (Brazil and Romania) and commercially available (China and Turkey).

study	Years old	IR (g day^{-1})	Index	Trace elements														HI
				Co	Cr	Cu	Fe	Mn	Ni	Pb	Se	Zn	Li	Mo	Cd	As	Al	
Brazil (current study)	8	0.61	CR	-	2.8×10^{-4}	-	-	-	-	-	-	-	-	-	-	-	-	
			HQ	0	1.9×10^{-1}	1.6×10^{-1}	5.6×10^{-2}	3.9×10^{-2}	0	0	0	5.2×10^{-1}	-	-	0	0	2.4×10^{-2}	9.9×10^{-1}
	18	1.46	CR	-	2.9×10^{-4}	-	-	-	-	-	-	-	-	-	-	-	-	
			HQ	0	1.9×10^{-1}	1.6×10^{-1}	5.6×10^{-2}	3.9×10^{-2}	0	0	0	5.2×10^{-1}	-	-	0	0	2.4×10^{-2}	9.9×10^{-1}
	30	1.65	CR	-	2.9×10^{-4}	-	-	-	-	-	-	-	-	-	-	-	-	
			HQ	0	1.9×10^{-1}	1.6×10^{-1}	5.6×10^{-2}	3.9×10^{-2}	0	0	0	5.2×10^{-1}	-	-	0	0	2.5×10^{-2}	9.9×10^{-1}
Romania (Petraru et al. 2021)	8	0.037	CR	-	3.6×10^{-4}	-	-	-	-	-	-	-	-	-	-	-	-	
			HQ	-	2.4×10^{-1}	7.0×10^{-3}	2.0×10^{-5}	7.9×10^{-3}	1.4×10^{-2}	-	3.3×10^{-1}	4.3×10^{-4}	1.4×10^{-1}	2.6×10^{-1}	-	-	-	9.9×10^{-1}
	18	0.08	CR	-	3.2×10^{-4}	-	-	-	-	-	-	-	-	-	-	-	-	
			HQ	-	2.2×10^{-1}	6.8×10^{-3}	2.0×10^{-5}	7.2×10^{-3}	1.2×10^{-2}	-	3.0×10^{-1}	3.9×10^{-4}	1.3×10^{-1}	2.3×10^{-1}	-	-	-	9.9×10^{-1}
	30	0.099	CR	-	3.5×10^{-4}	-	-	-	-	-	-	-	-	-	-	-	-	
			HQ	-	2.4×10^{-1}	7.4×10^{-3}	2.0×10^{-5}	7.9×10^{-3}	1.3×10^{-2}	-	3.3×10^{-1}	4.2×10^{-4}	1.4×10^{-1}	2.5×10^{-1}	-	-	-	9.9×10^{-1}
China (Zhu et al. 2011)	8	0.419	CR	-	-	-	-	-	-	1.4×10^{-6}	-	-	-	-	2.1×10^{-5}	2.7×10^{-5}	-	-
			HQ	-	-	2.7×10^{-2}	6.7×10^{-1}	5.1×10^{-2}	2.7×10^{-2}	4.0×10^{-2}	-	6.6×10^{-2}	-	-	5.7×10^{-2}	5.9×10^{-2}		9.9×10^{-1}
	18	0.99	CR	-	-	-	-	-	-	1.4×10^{-6}	-	-	-	-	2.1×10^{-5}	2.6×10^{-5}	-	
			HQ	-	-	2.6×10^{-2}	6.7×10^{-1}	5.1×10^{-2}	2.7×10^{-2}	3.9×10^{-2}	-	6.5×10^{-1}	-	-	5.6×10^{-2}	5.9×10^{-2}	-	9.9×10^{-1}
	30	1.128	CR	-	-					1.4×10^{-6}	-	-	-	-	2.1×10^{-5}	2.7×10^{-5}	-	
			HQ	-	-	2.7×10^{-2}	6.7×10^{-1}	5.1×10^{-2}	2.7×10^{-2}	4.0×10^{-2}	-		-	-	5.7×10^{-1}	5.9×10^{-2}	-	9.9×10^{-1}
Turkey (Mendil et al. 2009)	8	0.0093	CR	-	-	-	-	-	-	3.0×10^{-5}	-	-	-	-	5.1×10^{-7}	-	-	
			HQ	0.0065	-	9.9×10^{-4}	5.4×10^{-2}	3.1×10^{-2}	-	9.0×10^{-1}	-	1.3×10^{-3}	-	-	1.3×10^{-3}	-	-	9.9×10^{-1}
	18	0.0224	CR	-	-	-	-	-	-	3.1×10^{-5}	-	-	-	-	5.2×10^{-7}	-	-	
			HQ	1.0×10^{-1}	-	1.5×10^{-2}	8.4×10^{-1}	4.8×10^{-3}	-	1.4×10^{-2}	-	1.3×10^{-3}	-	-	2.1×10^{-8}	-	-	9.9×10^{-1}
	30	0.0253	CR	-	-	-	-	-	-	3.1×10^{-5}	-	-	-	-	5.2×10^{-7}	-	-	
			HQ	6.5×10^{-3}	-	9.9×10^{-3}	5.4×10^{-2}	3.1×10^{-2}	-	9.0×10^{-1}	-	2.0×10^{-2}	-	-	1.4×10^{-3}	-	-	9.9×10^{-1}
Turkey (Bakircioglu et al. 2013)	8	0.0097	CR	-	1.3×10^{-3}	-	-	-	-	3.2×10^{-7}	-	-	-	-	8.1×10^{-6}	-	-	
			HQ	-	8.8×10^{-1}	2.8×10^{-2}	4.8×10^{-3}	-	3.2×10^{-2}	9.3×10^{-3}	-	7.9×10^{-3}	-	-	2.1×10^{-2}	-	-	9.9×10^{-1}
	18	0.0234	CR	-	1.3×10^{-3}	-	-	-	-	3.2×10^{-7}	-	-	-	-	8.2×10^{-6}	-	-	
			HQ	-	8.9×10^{-1}	2.8×10^{-2}	4.8×10^{-3}	-	3.3×10^{-2}	9.4×10^{-3}	-	7.9×10^{-3}	-	-	2.2×10^{-2}	-	-	9.9×10^{-1}
	30	0.0264	CR	-	1.3×10^{-3}	-	-	-	-	3.2×10^{-7}	-	-	-	-	8.2×10^{-6}	-	-	
			HQ	-	8.9×10^{-1}	2.8×10^{-2}	4.8×10^{-3}	-	3.3×10^{-2}	9.4×10^{-3}	-	7.9×10^{-3}	-	-	2.2×10^{-2}	-	-	9.9×10^{-1}

2.5. CONCLUSION

This sunflower oil demonstrated optimal qualitative proprieties for consumption, correlated with observed results on fatty acids composition, physicochemical proprieties, thermal and oxidative qualities, optical proprieties, and trace elements comparison with DRIs/AI and FAO/WHO parameters herein evaluated. However, Cr concentration in sunflower oil was above FAO/WHO limits, which can be used as an indicator of ambience pollution.

However, to obtain values HI < 1, and CR < 10^{-4} , the maximum sunflower oil daily consumption varied between 0.61, 1.46, and 1.65 g kg^{-1}, respectively to the individuals aged 8, 18, and 30 years old. Moreover, the calculated results based on trace elements concentration regarding HI < 1, and CR < 10^{-4} indices of the sunflower oil previously qualitative approved show lower daily intake compared with prior daily consumption varying 25 to 142 g kg^{-1} day^{-1}.

Thus, it is expected that this quantitative daily consumption of sunflower oil here presented can be used for other vegetable oils and several foodstuffs for health improvement and metabolic diseases regulation.

2.6. GENERAL CONCLUSIONS AND FUTURE PERSPECTIVES

The Cerrado natural soil featured by high amount of aluminium (Al) and low amount of several others chemical elements such phosphorus (P) and zinc (Zn), iron (Fe), vanadium (V), chromium (Cr), barium (Ba), lead (Pb), nickel (Ni), copper (Cu), molybdenum (Mo), cadmium (Cd). Owing to this Cerrado soil topography, periodic corrections with biosolid and fertilizer are necessary, as well as, plenty use of farm pesticides to guarantee productivity.

Among several chemical elements As, Pb and Cr were quantified high in the guavira pulp collected to 500 m of the roadside that experienced high large vehicle traffic, which can be correlated with petroleum and diesel combustion from the engine, brake lining and tire wear, exposing these heavy metals and metalloids in the environment, which contaminated the soil and plants growing near of the roads.

In addition, As, Pb and Cr were determined high in guavira pulp collected near the farm with a distance of 3000 m to the road, which can be explained by the intensive utilization of the inorganic fertilizers to improve agricultural soils, such as biosolid and commercial phosphate fertilizers. Moreover, As was quantified majorly in Glyphosate-based herbicides, such as R Weather Max, Clinic EV, R3+, and Radical Tech+. Thus, As, Pb and Cr were observed high in fungicides (Folpan, Eyetak, Pictor and Teldor), herbicides (Matin and Starane) and insecticides (pyrinex) formulations, which appeared 5 to 100 times compared with recommended limits.

Although, As, Pb and Cr were quantified lower in the bush compared with the amount observed in guavira pulp collected near the roadside and farm, their amounts exceeded the FAO/WHO standard, as well as for 400 g daily consumption of fruits and vegetables recommended.

Therefore, among these three chemical elements above reported, As is the primary crucial heavy metal contaminant to fruit berry found in the areas experienced with intensive modern agriculture farming and road with a high vehicle traffic.

Thus, to minimize a possible occurrence of cancer risks caused by heavy metals, we calculated the projection to reduce the possible incidence of several metabolic diseases including cancers and recommend the consumption quantities ≤ 1 g day^{-1} to fruit berry pulp.

Although in literature vegetable oils daily consumption is reported from 25 to 142 g kg^{-1}, however, based on heavy metals and metalloids (trace elements) quantified in this sunflower oil obtained utilizing seed with hull using a domestic extraction machine was calculated that the suitable quantity of sunflower oil consumption varies according to individuals aged 8, 18, and

30 years shall be deemed 0.61, 1.46, and 1.65 g kg^{-1}, respectively, corresponding < 2 g day^{-1}, for attending HI = 0.99, and CR < 10^{-4}.

Therefore, other parameters were used to qualify this sunflower oil such as fatty acids composition, physicochemical optical features, thermal and oxidative qualities, which demonstrated that this oil is suitable for human consumption.

In this thesis, we want to highlight that chromium (Cr) chemical element concentrations were quantified with higher than FAO/WHO limits in guavira pulp and sunflower oil collected from plants of the Brazilian Cerrado biome marked by severe anthropogenic activities, which Cr concentrations can be used as indicators of ambient pollution.

Thus, it is expected that this quantitative daily consumption of guavira pulp and sunflower oil here presented can be used for several foodstuffs for health improvement and metabolic disease regulation.

From this perspective will be necessary to conduct studies for quantifying minerals in other species of fruits berry and oil-cultivated plants, soil, water, and dust of the region that are severely facing anthropogenic disturbance.

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ANNEXURES

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Article

High Concentration of Heavy Metal and Metalloid Levels in Edible *Campomanesia adamantium* Pulp from Anthropic Areas

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Abstract: This study aimed to quantify the extent of heavy metal, non-metal and metalloid levels in the *Campomanesia adamantium* pulp obtained from an area crossed by road experiencing high large vehicle traffic and intensive agriculture modern farming, to monitor the health risks associated with pulp consumption by humans. For this purpose, in three spots located within this area, ripe fruits were collected on the roadside, bush and farm-margin. Pulp samples were digested by microwave-assisted equipment, and chemical elements were quantified by ICP OES. The concentrations of K, Pb, Se, Fe, Mo, Zn, Co, Ni and Mn in the pulp collected in roadside/bush points showed statistical differences ($p < 0.05$). The heavy metals and metalloid concentrations that exceeded FAO/WHO standards were ordered Pb > As > Mo > Co > Ni > Mn > Cr. Therefore, among these metalloid and heavy metals, As, Pb and Cr were found to be higher in farm-margin > roadside > bush (1.5×10^{-3}, 1.1×10^{-3} and 6.2×10^{-4}), respectively. Therefore, As is the most important metalloid with higher levels in farm-margin, roadside and bush (1.5×10^{-3}, 1.0×10^{-3} and $6.0 \times 10^{-4} > 10^{-6}$–$10^{-4}$ and 3.33, 2.30 and 1.34 > 1), respectively, to total cancer risk and hazard quotient, if 10 g daily of pulp are consumed.

Keywords: Cerrado; myrtaceae; edible fruit; farm-margin; roadside; macro- and microelements; health risk

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1. Introduction

The relationship between anthropic activities and native fruits is extremely important for food security, including the role of metalloids and heavy metals in the contamination of land, water, and edible plants, which has been regarded as an environmental and public health hazard [1]. Due to severe anthropogenic activities, as high large vehicle traffic and intensive modern agriculture, the environment becomes prone to high toxicity and the bioaccumulation of heavy metals in plants used for food or medicines [2–4]. Among several species of plants, *Campomanesia adamantium* (Cambess.) O. Berg (Myrtaceae), popularly known as "Guavira or Guabiroba", stands out for its wide occurrence in the Cerrado and other biomes, such as those of the Atlantic Forest and Pampa in Brazil, which have intensive and intense anthropogenic activities [5]. In addition, roots, leaves and fruits of this species are popularly used as antirheumatic, antidiarrheal, hypocholesterolemic, anti-inflammation, urethritis and cystitis remedies, among other functions [6–8]. Moreover,

several studies have reported the potential activities of *C. adamantium* fruits as antibacterial and antifungal [9,10], anti-hyperalgesic, antidepressive [11], antimicrobial [12], antiproliferative against cancer cells [13,14], hepatoprotective [15], as an inhibitor of leukocyte mobility, neurogenic pain and oedema [7]. The genus *Campomanesia* includes 37 species, 26 of which are endemic in Brazil [5]. The *C. adamantium* fruits, characterized by a citrus aroma and sweet flavor, are consumed fresh or used to produce homemade liqueurs, juices, ice creams, jellies, backer products, and others [16]. Additionally, they are natural sources of a considerable amount of ascorbic acid, fibers, vegetable oil, polyphenols, and monoterpene substances [7,14,17].

To date, there are only studies that have quantified minerals in the peel, pulp and seed of *C. adamantium* collected near urban areas [18,19]. However, no studies have been carried out to assess the chemical elements in fruits collected close to roads with high vehicle traffic in agricultural regions. Fertilizers, pesticides, and vehicle fumes contain heavy metals and metalloids, such as potassium (K), arsenic (As), iron (Fe), lead (Pb), chromium (Cr), manganese (Mn), molybdenum (Mo), nickel (Ni), and other elements, which in high amounts contaminate the environment, edible plants, and consequently, humans [2,4,20,21].

In this context, using inductively coupled plasma optical emission spectroscopy (ICP OES), this study aimed to quantify potassium (K), lead (Pb), phosphorus (P), arsenic (As), selenium (Se), iron (Fe), molybdenum (Mo), zinc (Zn), cobalt (Co), nickel (Ni), manganese (Mn), and chromium (Cr) in the *C. adamantium* fruit pulp collected in these three spots from the roadside (500 m) to bush (1000 m) and farm-margin (3000 m), marked by intense anthropogenic activities. The concentrations of these chemical elements were compared to the recommended tolerable maximum intake levels established by Dietary Reference Intakes (RDI) for children aged 4–8 years, adults and pregnancy (31–50 years), and the Food Agriculture Organization of the United Nations (FAO) and World Health Organization (WHO) parameters for human intake. According to the Food and Drug Administration (FDA) parameters, the contents of the chemical elements in this pulp were qualified as a good source, excellent source or no good source. To the best of our knowledge, this is the first report on high concentrations of metalloids such as As and heavy metals like Pb and Cr in the pulp of a wild edible plant collected near high vehicle traffic and farming with the intensive use of fertilizers and pesticides. According to the carcinogenic risk calculated to health risk assessment, we propose that individuals consume 1 g/day instead of the 400 g/day—as recommended by WHO for edible fruits and vegetables—due to the high concentrations of As associated with several types of cancer and other diseases.

2. Materials and Methods

2.1. Fruit Collection and Sample Preparation

Ripe fruits were collected in twenty-one different points, separated from each other by 20 m. The fruits were mixed according to the collected distance from the roadside (500 m) to the bush (1000 m) and farm-margin (3000 m) in Campo Grande, Mato Grosso do Sul state, Brazil, 20°46′34.208″ S, 54°10′28.567″ W (Figure 1), in November 2019. Manually, the pulp was separated from the peel and seed, immediately dried in an air circulation oven at 40 °C for 48 h. The dried pulp was milled using mortar and pestle and sieved to obtain the refined powder, placed into an amber and hermetic glass bottle and frozen at −20 °C for further analyses.

The *C. adamantium* was registered in System of Genetic Resource Management and Associated Traditional Knowledge (SisGen) of the Ministry of the Environment (registration number A7716EC).

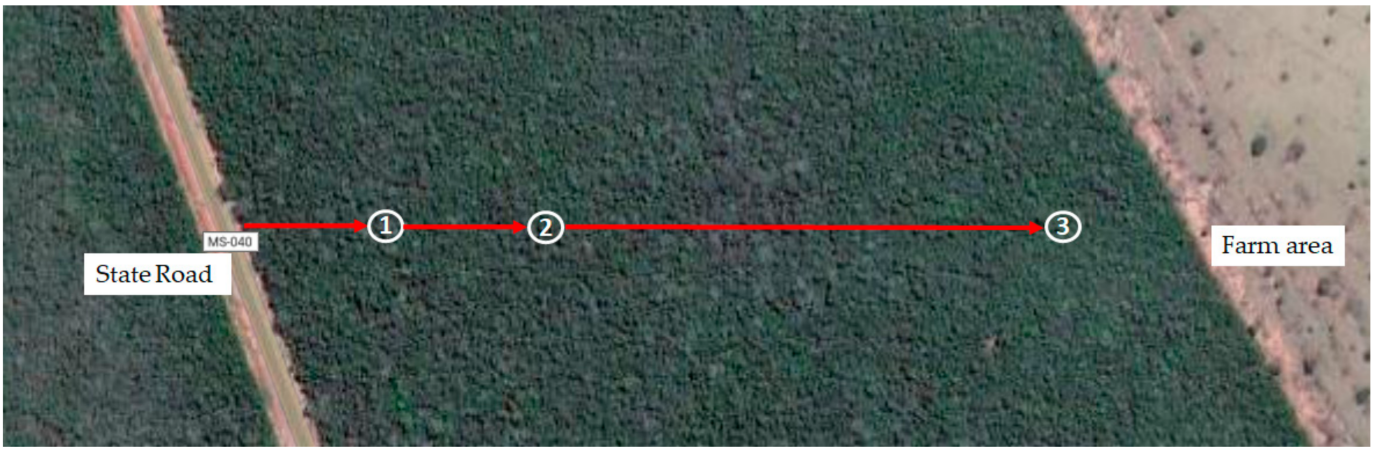

Figure 1. Collection spots of *Campomanesia adamantium* fruits located between the state road MS-040 with high large vehicle traffic and intensive modern agriculture in Campo Grande—Mato Grosso do Sul State, Brazil. 1. roadside = 500 m; 2. bush = 1000 m; and 3. farm-margin = 3000 m.

2.2. Microwave-Assisted Digestion Procedure

The pulp samples were weighed according to Lima et al. [19] and prepared as described: 0.5 g sample plus 5 mL HNO_3 (65% Merck, Darmstadt, Germany) and 3 mL H_2O_2 (35% Merck, Darmstadt, Germany) were individually placed into PTFE bottles of the DAP 60 type (Berghof). The mixture was allowed to remain in the open air for 10 min predigestion and then digested using a microwave digestion system (Speedwave four®, Berghof, Germany). After the microwave system's digestion procedure, the samples were transferred from the vessels to 50 mL Falcon vessels and which were then filled to 30 mL with ultrapure water (conductivity 18.2 MΩcm (Millipore), Biocel, Germany). The samples were digested in the microwave system according to the schedule shown in Table 1. All the digestion analysis steps were performed in triplicate.

Table 1. Microwave digestion parameters.

	Steps			
	1	**2**	**3**	**4**
Power (W)	1305	1305	0	0
Temperature (°C)	170	200	50	50
Ramp time (min)	1	1	1	1
Hold time (min)	10	15	10	1
Pressure (Bar)	35	35	0	0

2.3. ICP OES Elemental Analysis

Chemical elements were quantified using the ICP OES (Thermo Fischer Scientific, Bremen, Germany, iCAP 6300 Duo) technique. The selected emission lines (wavelength in nm) for determining elements in pulp and operating conditions of ICP OES are summarized in Table 2.

2.4. Calibration Curves

For the ICP OES, standard solutions for analytical calibration were prepared by diluting a standard multiple-element stock solution containing 1000 mg/L of the Al, As, Ca, Cd, Co, Cr, Cu, Fe, Mg, Mn, Mo, Na, Ni, P, S, V, Se, and Zn from SpecSol (SpecSol, Quimlab, Brazil). For each element detected, the limit of detection (LOD) of 0.0002–0.003 (mg/L), the limit of quantification (LOQ) of 0.006–0.01 (mg/L) and the correlation coefficient (R^2) of 0.9995–0.9998 were determined. One blank and seven calibration curves were generated using the following concentrations: 0.01, 0.02, 0.05, 0.2, 1.0, 2.0 and 5.0 mg/L of the

element standard. All experiments were carried out in triplicate. The detection limit (LOD) was calculated as three times the standard deviation of the blank signal (B) expressed in concentration divided by the slope of the analytical curve (AC): LOD = 3*B/AC, and the limit of quantification (LOQ) was obtained as ten times the standard deviation of the blank divided by the slope of the analytical curve: LOQ = 10*B/AC [22].

An addition/recovery test for the elements under study was performed in a pulp sample by spiking 0.5 mg/L of each analyte. The method had a recovery interval of 80%–110% for the spike 0.5 mg/L, which was found to be between 70% and 120% to the established limit proposed by the Union of Pure and Applied Chemistry (IUPAC) and Association of Official Analytical Chemists (AOAC) [23,24].

Table 2. Instrumental analytical conditions for the ICP OES of element analysis.

Parameters	Setting
RF power (W)	1250
Sample flow rate (L mn^{-1})	0.45
Plasma gas flow rate (L mn^{-1})	12
Integration time (s)	5
Stabilization time (s)	20
Pressure of nebulization (p si)	20
Plasm view	Axial
Gas view	Air
Analytical wavelength (nm)	Fe (259.940), Ni (231.604), Co (228.616), Cr (267.716), As (193.759), Pb (214.441), Mo (202.030), Mn (257.610), P (177.595), K (766.490), Zn (213.856), Se (196.090).

2.5. Human Health Risk Assessment

The results of the concentrations of the chemical elements were compared with recommended intake standards of the RDA/AI, UL, FAO/WHO, USEPA and hazard quotient. The human risk for a non-carcinogenic was calculated following the equation adopted by Liang et al. [25]. Cancer risk was the probability of an individual developing any cancer over a lifetime, during the daily doses exposure to 70 years; the chronic daily intake dose (CDI) of carcinogenic elements (mg/kg/day); and slope factor (SF) was the carcinogenicity (mg/kg/day). The SFs of As, Cr and Pb are 1.5, 0.5 and 0.0085 mg/kg/day, respectively, following Equation (1):

$$\text{Cancer Risk} = \text{CDI} \times \text{SF} \quad (1)$$

The cancer risk is a sum of individual variety carcinogenic elements risk in different exposure pathways, which is the total cancer risk (R). In agreement with USEPA [26], the value of acceptable or tolerable cancer risk ranges from 10^{-6} to 10^{-4}, while > 10^{-4} is considered unacceptable.

The human health risk of heavy metal intake was evaluated based on the chronic daily intake dose (CDI, mg/kg/day) for a chemical contaminant in the pulp over the exposure period and the pulp intake quantity. CDI was calculated using the following Equation (2):

$$\text{CDI}_{\text{pulp}} = \frac{\text{C}_{\text{pulp}} \times \text{IR}_{\text{pulp}} \times \text{EF} \times \text{ED}}{\text{BW} \times \text{AT}} \quad (2)$$

where CDI_{pulp}—chronic daily pulp intake dose; C_{pulp}—concentration of chemical element content in the pulp; IR_{pulp}—ingestion rate (mg/day); EF—exposure frequency (90 days available/year); ED—exposure duration (life expectancy = 70 years); BW—body weight; and AT—average time (ED × 365 days). The adult's body weight, approximately 70 kg, and the average daily pulp consumption was 10 g/day. The risk to human health by the intake of heavy metal-contaminated pulp was measured using a hazard quotient (HQ),

which is a ratio of CDI and chronic oral reference dose (RfD), determined by the following Equation (3):

$$HQ = \frac{CDI}{RfD} \quad (3)$$

The RfD values for the risk calculation were established by the Joint Food and Agriculture Organization/World Health Organization Expert Committee on Food Additives [27] and the United States Environmental Protection Agency [28]. The RfD values for the elements were established: K = not available; Pb = 0.004 mg/kg bw/day; P = not available; As = 0.0003 mg/kg bw/day; Se = not available; Fe = 0.7 mg/kg bw/day; Mo = 0.005 mg/kg bw/day; Zn = 0.3 mg/kg bw/day; Co = 0.03 mg/kg bw/day; Ni = 0.02 mg/kg bw/day; Mn = 0.14 mg/kg bw/day; and Cr = 0.003 mg/kg bw/day [28]. As shown in Equation (3), a toxic risk is considered to occur if HQ > 1, whereas HQ < 1 represents a negligible hazard (adverse non-carcinogenic effects).

2.6. Statistical Analysis

The data were analyzed by one-way ANOVA using the GraphPad Prism software version 8.0 for Mac (GraphPad Software, San Diego, CA, USA). The significance of the differences between the means for the individual element level was considered at $p < 0.05$.

3. Results and Discussion

In this section, the article was composed of two Sections: Section 3.1 present data on the concentration of the chemical elements obtained in pulp collected in roadside, bush and farm-margin, and the comparison of these concentrations with other published studies. In Section 3.2, data of the type of chemical elements quantified for each site was used to calculate EDI and HQ values.

3.1. The Chemical Element Concentration in Pulp Collected in Three Different Sites

Twelve chemical elements were found in *C. adamantium* pulp collected in three different sites from the road: roadside (500 m); bush (1000 m); and farm-margin (3000 m) (Table 3).

Table 3. *Campomanesia adamantium* pulp collected in three different sites from the road: roadside (500 m); bush (1000 m); and farm-margin (3000 m), quantified by ICP OES (mg/100 g ± SD) compared with nutritional recommendations for adult, pregnancy and children by RDA/AI, UL and FAO/WHO.

Elements	Roadside (mg/100 g)	Bush (mg/100 g)	Farm-Margin (mg/100 g)	Male 31–50 y RDA/AI * (mg/day)	Female 31–50 y RDA/AI * (mg/day)	Male/ Female 31–50 y UL (mg/day)	Pregnancy 31–50 y		Children 4–8 y		FAO/WHO (mg/day)
							RDA/AI * (mg/day)	UL (mg/day)	RDA/AI * (mg/day)	UL (mg/day)	
K	33.02 ± 0.01	31.02 ± 0.01	58.01 ± 1.34	4700	4700	ND	4700	ND	4700	ND	3510 [29]
Pb	5.36 ± 0.02	7.02 ± 0.01	6.85 ± 1.05	ND	ND	ND	ND	ND	ND	ND	0.02 [30]
P	3.24 ± 0.02	3.04 ± 0.02	5.24 ± 0.80	700	700	4000	700	3500	500	3000	700 [29,31]
As	1.96 ± 0.04	1.14 ± 0.03	2.84 ± 0.52	ND	ND	ND	ND	ND	ND	ND	0.01 [30]
Se	0.20 ± 0.01	0.22 ± 0.02	0.40 ± 0.10	55	55	400	400	60	30	150	0.06 [31]
Fe	0.23 ± 0.02	0.12 ± 0.01	0.40 ± 0.10	8	18	45	27	45	10	40	2.00 [32]
Mo	0.10 ± 0.02	0.09 ± 0.02	0.19 ± 0.01	150	150	1100	50	2000	22	600	0.045 [31]
Zn	0.08 ± 0.01	0.07 ± 0.01	0.13 ± 0.02	11	8	40	11	40	5	12	3.00 [31]
Co	0.07 ± 0.01	0.02 ± 0.00	0.08 ± 0.02	ND	ND	ND	ND	ND	ND	ND	0.0014 [33]
Ni	0.06 ± 0.01	0.04 ± 0.01	0.10 ± 0.02	ND	ND	1	ND	1	ND	0.3	0.20 [32]
Mn	0.05 ± 0.01	0.03 ± 0.01	0.07 ± 0.01	2.30	1.80	11	2.60	11	1.50	3	3.00 [31]
Cr	0.03 ± 0.01	0.01 ± 0.00	0.05 ± 0.01	0.035 *	0.025 *	ND	0.030 *	ND	0.015 *	ND	0.002 [32]

Note. ND—not determined; * The value for AI is used when there are no calculated values for the RDA.

The concentration of chemical elements quantified in *C. adamantium* pulp samples is depicted in decreased order in Table 3. The average concentration of chemical elements in pulp collected on roadside followed in decreased order K > Pb > P > As > Fe > Se > Mo > Zn > Co > Ni > Mn > Cr; bush: K > Pb > P > As > Se > Fe > Mo > Zn > Ni > Mn > Co > Cr, and farm-margin: K > Pb > P > As > Se > Fe > Mo > Zn > Co > Ni > Mn > Cr. The concentrations of Pb, As and Cr in the present study are higher compared with the average reported for fruits and pulp collected in areas with a lower exposure to contaminants produced by anthropogenic activities [18,19,34], that exceed the FAO/WHO permissible limit recommended for edible berries and small fruits [29–33]. On the other hand, high concentrations of Mo, Co, Ni and Mn were reported in *C. adamantium* fruits compared with the present study [34], which could be correlated with the occurrence of these chemical elements in natural environments [35,36].

In general, the average of all chemical elements quantified in *C. adamantium* pulp followed a decreasing order K > Pb > P > As > Se > Fe > Mo > Zn > Co > Ni > Mn > Cr. The one-way ANOVA test values considering the concentrations of each element were in collected in the three sites; then, we compared the pairs roadside/bush, roadside/farm-margin and bush/farm-margin. The concentrations of K, Pb, Se, Fe, Mo, Zn, Co, Ni and Mn in the pulp collected in roadside/bush points showed statistical differences ($p < 0.05$). However, significant differences ($p > 0.05$) were not observed when comparing the concentration of each chemical element found in *C. adamantium* pulp collected in roadside/bush/farm-margin.

Thus, it was observed that the concentration behavior of chemical elements decreased from the roadside (500 m) to bush (1000 m) and increased to farm-margin (3000 m). However, the concentrations of Pb and Se increased from the roadside to the bush and more toward the farm-margin, as illustrated in Figure 2.

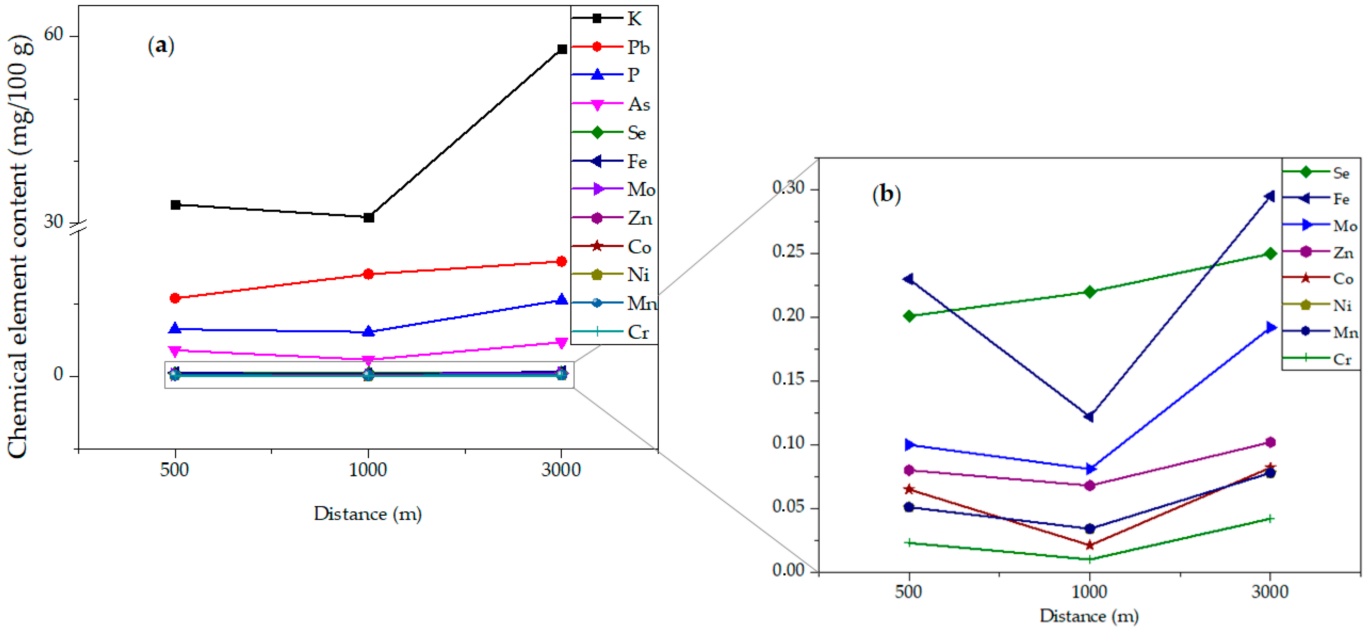

Figure 2. Behavior of the chemical elements' quantities distribution in *Campomanesia adamantium* pulp collected in three different sites: roadside (500 m); bush (1000 m); and farm-margin (3000 m), quantified by ICP OES (mg/100 g): (**a**) chemical element content >1 mg/100 g; (**b**). chemical element content ≤0.4 mg/100 g.

Table 3 list the levels of chemical elements quantified (mg/100 g ± SD) in the *C. adamantium* pulp compared with the limit specification of RDAs/AI and UL values for males and females (31–50 y), pregnant women (31–40 y) and children (4–8 y) [37], and FAO/WHO and WHO [29–33] permissible levels for fruits and food.

The percentages of chemical elements in the pulp were calculated from the mean values (Table 3) based on RDA, AI, UL, and FAO/WHO and WHO limits [29–33], while

the studied chemical elements were qualified based on the FDA parameters (10%–19% for "good source" of nutrition, and ≤20% for "excellent source") [38].

Potassium (K) concentrations in roadside (33.02 ± 0.01 mg/100 g), bush (31.02 ± 0.01 mg/100 g) and farm-margin (58.01 ± 1.34 mg/100 g) pulp correspond to proportions ≤ 1% for males, females, pregnant women, and children to 4700 mg/day by RDA parameters. The UL of K has no established values for males, females, pregnant women and children. The K content in this pulp was the lowest (3510 mg/day) FAO/WHO limit [29]. According to FDA parameters, this pulp is not a good source of K [38]. The K concentrations in this pulp are lower than 130–253 mg/100 g, as reported in previous studies for *C. adamantium* fruits and pulp [18,19,34], which can be explained by the higher levels of metalloid and heavy metals observed in this pulp which can reduce K content, such as Cr, which results from intense anthropogenic activity [39]. However, K concentrations in this pulp are near blueberry and alfalfa (39 mg/100 g) [38]. The health benefit of K in the body is associated with blood pressure regulation, stroke risk reduction, preventing renal system dysfunction, decreasing urinary calcium excretion, reducing kidney stone formation and osteoporosis disease [40], regulating blood lipid concentrations [36] and maintaining bone and cardiovascular health [41–43].

Lead (Pb) concentrations in roadside (5.36 ± 0.02 mg/100 g), bush (7.02 ± 0.01 mg/100 g) and farm-margin (7.88 ± 1.05 mg/100 g) pulp correspond to 26,800%, 35,100% and 39,480% by 0.02 mg/day FAO/WHO parameters [30]. The RDA and UL parameters for Pb have no established values for adults and children. Based on the FDA parameters, this pulp is an excellent source of Pb [38]. In this pulp, Pb concentrations are lower than 0.06 mg/100 g, as reported in previous studies for fruits of *C. adamantium* [18]. On the other hand, Pb contents in this pulp are near those of other edible fruit such as apple (2.35 mg/100 g), mango (6.72 mg/100 g) [44] and tomato (5.41–11.73 mg/100 g) [45]. The risk of consuming food with a high amount of Pb is correlated with intelligence reduction, bone joint weakness, accelerated bone maturation, increased blood pressure, spontaneous abortion, renal dysfunction, allergic diseases [46], respiratory and cardiovascular diseases [47].

Phosphorus (P) concentrations in roadside (3.24 ± 0.02 mg/100 g), bush (3.04 ± 0.02 mg/100 g) and farm-margin (5.24 ± 0.8 mg/100 g) pulp correspond to proportions ≤ 1% for males, females and pregnant women (700 mg/day) and children (500 mg/day) by RDA parameters. The P contents correspond to values ≤ 0.2% for males/females (4000 mg/day), pregnant women (3500 mg/day) and children (3000 mg/day) by UL limits. The P concentrations of the roadside, bush and farm-margin pulp correspond to proportions < 1% to 700 mg/day by FAO/WHO limits [29,31]. According to FDA parameters, this pulp is not a good source of P [31]. Indeed, P concentrations in this pulp are lower than 17–196 mg/100 g reported in previous studies on fruits and pulp of *C. adamantium* [18,19,34]. However, P concentrations in this pulp are near of blackberry and watermelon (5–11 mg/100 g) [38]. The health benefit of P consumption is related to bone mineralization, cell energy generation, cardiovascular regulation and neuromuscular function [48], and the modulation of short-chain fatty acid gut bacteria producers [49].

Arsenic (As) concentrations in roadside (1.96 ± 0.04 mg/100 g), bush (1.14 ± 0.03 mg/100 g) and farm-margin (2.84 ± 0.52 mg/100 g) pulp correspond to 19,600%, 11,400% and 28,400% by 0.01 mg/day FAO/WHO limits [30]. The RDA and UL parameters for As have no established values for adults and children. By FDA parameters, this pulp is an excellent source of As [38]. The As contents in this pulp are higher than 0.07 mg/100 g, as reported in previous studies on *C. adamantium* fruits [18] and are near those of edible vegetables such as lettuce (2.73 mg/100 g) [8], *Colocasia antiquorum* (0.6–12.5 mg/100 g), gourd leaf (0.8–15.8 mg/100 g) [50], fish, seafood and seafood products (0.16–0.56 mg/100 g) [51]. The risk of the consumption of food with a high amount of As is associated with cancers (skin, lung and bladder) [50], respiratory disease, gastrointestinal disorder, liver malfunction, neuro–cardiovascular dysfunction, anemia disorder, leucopenia and thrombocytopenia effects, diabetes [52], cytotoxicity and genotoxicity effects [53].

Selenium (Se) concentrations in roadside (0.20 ± 0.01 mg/100 g), bush (0.22 ± 0.02 mg/100 g) and farm-margin (0.40 ± 0.1 mg/100 g) pulp correspond to values <1% for males and females (55 mg/day), pregnant women (400 mg/day) and children (30 mg/day) by RDA parameters. The Se contents in the roadside, bush and farm-margin pulp correspond to proportions of <1% for males and females (400 mg/day), pregnant women (60 mg/day) and children (150 mg/day) by UL limits. The Se concentrations in roadside, bush and farm-margin pulps correspond, respectively, to 333.33%, 366.67% and 500% by 0.06 mg/day FAO/WHO limits [31]. According to FDA parameters, this pulp is an excellent source of Se [38]. The Se concentrations in this pulp are lower than the amount of 0.88 mg/100 g reported in previous studies on *C. adamantium* fruits [18], and higher than that reported in grapes, apricot, kiwi, litchi, macadamia and pistachio (0.0001–0.007 mg/100 g) and near that of the cashew nut (0.02 mg/100 g) [38]. Other studies have recommended 0.018 mg/day of Se quantity intake for children (4–6 y), 0.023 mg/day for adolescent males 10–18 y and 0.021 mg/day for adult females (19–65 y), 0.027 mg/day for males and 0.0204 mg/day [54]. The benefit of the consumption of Se is correlated with preventing and decreasing diabetes mellitus, cancers [55], improving male fertility [56,57], human neuropathies [58] and hepatic steatosis [59].

Iron (Fe) concentrations in roadside (0.23 ± 0.02 mg/100 g), bush (0.12 ± 0.01 mg/100 g) and farm-margin (0.40 ± 0.10 mg/100 g) pulp correspond to values <4% by RDA parameters for males (8 mg/day), females (18 mg/day), pregnant women (27 mg/day) and children (10 mg/day). The Fe contents in the roadside, bush and farm-margin pulp correspond to <1% by UL parameters for males, females and pregnant women (45 mg/day) and children (40 mg/day). In concordance with FDA parameters, this pulp is not a good source of Fe [38]. The Fe concentrations in this pulp are lower than the amount of 1–2.6 mg/100 g reported in previous studies on fruits and pulp of *C. adamantium* [18,19,34]. However, the Fe content of this pulp is between that of apple, guava and pineapple (0.12–0.29 mg/100 g) [38]. The health benefits of food consumption with Fe are improving maximal oxygen respiration and exercise performance, hemoglobin synthesis, electron transport, anemia prevention, deoxyribonucleic acid synthesis, gut microbiota modulation, neurodevelopment, immunity, pregnancy development [60–62].

Molybdenum (Mo) concentrations in roadside (0.10 ± 0.02 mg/100 g), bush (0.09 ± 0.02 mg/100 g) and margin-farm (0.19 ± 0.01 mg/100 g) pulp correspond to proportions ≤1% by RDA parameters for males and females (150 mg/day), pregnant women (50 mg/day) and children (22 mg/day). The Mo contents in the roadside, bush and farm-margin pulp correspond to values ≤0.2% by UL parameters for males and females (1100 mg/day), pregnant women (2000 mg/day) and children (600 mg/day). The Mo concentrations in roadside, bush and farm-margin pulp correspond, respectively, to 222.22%, 177.78% and 422.22% by 0.045 mg/day FAO/WHO parameters [31]. In agreement with FDA parameters, this pulp is an excellent source of Mo [38]. However, the Mo concentrations in this pulp are lower than the amount of 0.4–30 mg/100 g reported in previous studies on fruits of *C. adamantium* [19,34]. The Mo food consumption is recommended for infants (0.015–0.04 mg/day) and all individuals ≥10 years old (0.025–0.15 mg/day) [63]. The health benefit of Mo is correlated with toxicity prevention by several metabolites, reduction in aerosol organs irritability, night blindness, neurological damage, aches and pain [64–66]. The Mo concentrations of this pulp are between those of pea seeds and tomato (0.10–0.20 mg/100 g) [67].

Zinc (Zn) concentrations in roadside (0.08 ± 0.01 mg/100 g), bush (0.07 ± 0.01 mg/100 g) and farm-margin (0.13 ± 0.02 mg/100 g) pulp correspond to values <2% by RDA limits for males and pregnant women (11 mg/day), females (8 mg/day) and children (5 mg/day). The Zn contents in the roadside, bush and farm-margin pulp correspond to 1% by UL parameters for males, females, pregnant women (40 mg/day) and children (12 mg/day). The Zn concentrations in this pulp correspond to 2.67%, 2.27% and 3.4% by 3 mg/day FAO/WHO limits [31]. Based on FDA parameters, this pulp is not a good source of Zn [31]. The Zn concentrations in this pulp are lower compared with the

amount of 0.2–0.5 mg/100 g reported in previous studies on fruits and pulp of *C. adamantium* [18,19,34]. However, the Zn amounts are between those of apple, grapes and tomato (0.04–0.17 mg/100 g) [38]. The health benefit of the consumption of Zn food is associated with preventing or reducing oxidative stress, infections (malaria, pneumonia and diarrhea), cell ageing, atherosclerosis, neuropsychological diseases, autoimmune and degenerative diseases, Alzheimer's disease, inflammation cytokine storms, cancers, diabetes mellitus, obesity, depression, gastrointestinal and reproductive organ dysfunction, retina disease, and improving fetal and childhood skeletal growth and development [68–70].

Cobalt (Co) concentrations in roadside (0.07 ± 0.01 mg/100 g), bush (0.02 ± 0.00 mg/100 g) and farm-margin (0.08 ± 0.02 mg/100 g) pulp correspond to 5000%, 1428.57% and 5714.29% by 0.0014 mg/day WHO limits [33]. The RDA and UL parameters for Co have no established value for adults and children. Conforming to FDA parameters, this pulp is an excellent source of Co [38]. The Co concentrations in this pulp are lower than 8 mg/100 g reported in previous studies on *C. adamantium* pulp [34]. The Co concentrations are between strawberries, apple, grapes, mango, tomato and orange (0.03–0.08 mg/100 g) [44]. The risk of consuming food with a high amount of Co is correlated with inflammation and hypersensitivity reactions [71], neurological, cardiovascular and endocrine deficiency [72].

Nickel (Ni) concentrations in roadside (0.06 ± 0.01 mg/100 g), bush (0.04 ± 0.01 mg/100 g) and (0.1 ± 0.02 mg/100 g) pulp correspond to 6%, 4% and 10% for males, females, pregnant women, and 20%, 13.33% and 33.33% for children, respectively, by 1 mg/day, 1 mg/day and 0.3 mg/day UL limits. The Ni concentrations of the roadside, bush and farm-margin correspond to 30%, 20% and 50% by 0.2 mg/day FAO/WHO limits [32], respectively. The RDA parameters for Ni has no established value for adults and children. According to FDA parameters, this pulp is an excellent source of Ni [38]. The Ni concentrations in this pulp are lower than 4.2 mg/100 g reported in previous studies on fruits of *C. adamantium* [18]. The Ni concentrations are between those of paw-paw, mango, watermelon and banana fruits (0.023–0.089 mg/100 g) [73]. Some articles reported that the health benefit of Ni food consumption is correlated with gut microbiota balance and welfare [74]. However, other studies correlated Ni with hazardous conditions for human health such as cardiovascular, kidney and lung dysfunctions and oxidative stress [75].

Manganese (Mn) concentrations in roadside (0.05 ± 0.01 mg/100 g), bush (0.03 ± 0.01 mg/100 g) and farm-margin (0.07 ± 0.01 mg/100 g) pulp correspond to values ≤4% for males (2.3 mg/day), females (1.8 mg/day), pregnant women (2.6 mg/day) and children (1.5 mg/day) by RDA parameters. The Mn contents correspond to proportions <2.5% for males/females and pregnant women (11 mg/day), and children (3 mg/day) by UL limits. The Mn concentrations in pulps of roadside, bush and farm-margin correspond to 1.33%, 1.00% and 2.33%, respectively, by 3 mg/day FAO/WHO limits [31]. By FDA parameters, this pulp is not a good source of Mn [38]. The Mn concentrations in this pulp are lower than the amounts of 0.09–0.21 mg/100 g reported in previous studies on fruits and pulp of *C. adamantium* [18,19,34]. However, the Mn contents are near those of paw-paw and wheat (0.08–1.0 mg/100 g) [76]. The health benefit of the consumption of Mn food is associated with gut microbiota balance, regulating oxygen species and anemia conditions between mother and fetus and neurodevelopment [77–79].

Chromium (Cr) concentrations in roadside pulp was 0.03 ± 0.01 mg/100 g, which corresponds to 116.67%, 83.33%, 100% and 50% for males (0.035 mg/day), females (0.025 mg/day), pregnant women (0.03 mg/day) and children (0.015 mg/day) by AI parameters, respectively. The Cr content of 0.01 ± 0.00 mg/100 g in bush pulp corresponds to 350%, 250%, 300%, and 150% for males, females, pregnant women and children, respectively, by AI limits. The Cr content in farm-margin pulp was 0.05 ± 0.01 mg/100 g, which corresponds to 70%, 50%, 60%, and 30% for males, females, pregnant women and children, respectively, according to the AI standard. The Cr concentrations in the roadside, bush and farm-margin pulp correspond to 6.67%, 20% and 4%, respectively, by 0.002 mg/day FAO/WHO limits [32]. The RDA and UL parameters for Cr have no established values for adults and children. According to FDA parameters, this pulp is a good source of Cr [38]. However,

the Cr concentrations in this pulp are lower than the amount of 0.1–1.14 mg/100 g reported in previous studies on *C. adamantium* pulp [19,34]. The Cr contents are near edible fruits such as strawberry and melon (0.3 mg/100 g) [80].

3.2. Health Risk Assessment

The carcinogenic risk (CR) was calculated for three chemical elements Pb, As and Cr in pulp obtained from fruits collected in roadside, bush and farm-margin areas (Table 4). The values of As and Cr were farm-margin > roadside > bush, while the Pb values differed (farm-margin > bush > roadside). The total cancer risk (R) values for farm-margin, roadside and bush were 1.5×10^{-3}, 1.1×10^{-3} and 6.2×10^{-4}, respectively, which were higher compared with the acceptable parameters (10^{-6} to 10^{-4}), showing the importance of these values in terms of their carcinogenic risk for *C. adamantium* pulp consumers of 10 g/kg/day [26]. The total cancer risk is presented in decreased order As > Pb > Cr, demonstrating that As is the main pollutant chemical element that can be correlated with several cancer incidences among all heavy metals found in this pulp. Furthermore, the total cancer risk incidence can be higher for those who consume the recommended intake of 400 g/day [81] of pulp from farm-margin, roadside and bush (6.1×10^{-2}, 4.2×10^{-2} and 2.5×10^{-2}, respectively), in the region crossed by a road of high large vehicle traffic and intensive modern agriculture. However, the total cancer risks for the consumption of 1 g/kg/day of pulp from the roadside, bush and farm-margin were estimated to 1.1×10^{-4}, 6.3×10^{-5} and 1.5×10^{-4}, respectively, which are near of within acceptable parameters [26].

Table 4. Carcinogenic risk (CR), chronic daily intake dose (CDI, mg/kg bw/day) and hazard quotient (HQ) of chemical elements based on 10 g of *Campomanesia adamantium* pulp collected at three different sites from the road: roadside (500 m), bush (1000 m) and farm-margin (3000 m).

Samples		K	Pb	P	As	Se	Fe	Mo	Zn	Co	Ni	Mn	Cr
Roadside	CR	-	0.000016	-	0.001036	-	-	-	-	-	-	-	0.0000053
	CDI	0.011631	0.001888	0.001141	0.000690	0.000070	0.000081	0.000035	0.000028	0.000025	0.000021	0.000018	0.000011
	HQ	-	0.472016	-	2.301370	-	0.000116	0.007045	0.000094	0.000822	0.001057	0.000126	0.003523
Bush	CR	-	0.000021	-	0.000602	-	-	-	-	-	-	-	0.0000018
	CDI	0.010927	0.002473	0.001071	0.000402	0.000077	0.000042	0.000032	0.000025	0.000007	0.000014	0.000011	0.000004
	HQ	-	0.618200	-	1.338552	-	0.000060	0.006341	0.000082	0.000235	0.000705	0.000076	0.001174
Farm-margin	CR	-	0.000024	-	0.001501	-	-	-	-	-	-	-	0.0000088
	CDI	0.020434	0.002779	0.001846	0.001000	0.000141	0.000141	0.000067	0.000046	0.000028	0.000035	0.000025	0.000018
	HQ	-	0.694814	-	3.334638	-	0.000201	0.013386	0.000153	0.000939	0.001761	0.001233	0.005871

The non-carcinogenic risks for chemical elements are summarized in Table 4. The CDI values of the chemical elements in fruit pulp were presented in decreased order for three collection sites: K > Pb > P > As > Fe > Se > Mo > Zn > Co > Ni > Mn > Cr for roadside, K > Pb > P > As > Se > Fe > Mo > Zn > Co > Ni > Mn > Cr for bush, and K > Pb > P > As > Se = Fe > Mo > Zn > Ni > Co > Mn > Cr for farm-margin. The ordered concentrations of chemical elements are different for Fe and Se from roadside, while these are Se, Fe, Ni and Co for the farm-margin compared with bush areas. The major chemical elements in the pulp in decreased order are farm-margin > roadside > bush, which signifies that the farm and road have spread these chemical elements to pollute fruits. In contrast, Pb and Se are ordered from farm-margin > bush > roadside, which explains that the highest amount of these chemical elements have spread from the farm.

The hazard quotient (HQ) values of the chemical elements in roadside pulp estimated in decreased order are As > Pb > Mo > Cr > Ni > Co > Fe > Mn > Zn; in bush pulp: As > Pb> Mo > Cr > Ni > Co > Fe > Zn > Mn; and in farm-margin pulp: As > Pb > Mo > Cr > Ni > Co > Mn > Fe > Zn. The contents of Mn, Fe and Zn are irregularly distributed in farm-margin, roadside and bush areas. The majority of chemical elements were ordered as farm-margin > roadside > bush, which explains that the farm and road are sources of higher amounts of these chemical elements. In contrast, Pb is ordered as farm-margin > bush > roadside, meaning that this chemical element has spread in a higher amount from

the farm. The majority of chemical elements presented HQ < 1, while the highest values of As in the farm-margin, roadside and bush were 3.33, 2.30 and 1.34, respectively. Therefore, with a consumption of 10 g/kg/day of pulp, As could be the main cause of several cancer types and other chronic diseases.

4. Conclusions

According to RDA and UL limits, the pulp of *C. adamantium* collected in areas located between the road subject to high large vehicle traffic and intensive modern agriculture farming presented the lowest concentration of K, P, Se, Fe, Mo, Zn, Ni, and Mn. However, based on FAO/WHO parameters, the highest concentrations are Pb, As, Se, Mo, Co and Ni, and the lowest are K, P, Fe, Zn and Mn. The Cr concentration is above FAO/WHO and AI limits. Values of Pb, As, Co and Cr are not established by RDA and UL standards, including K, which are not established for the last parameter. This pulp is an excellent source of Pb, As, Se, Mo, Co, Ni and Cr, while it is not a good source of K, P, Fe, Zn and Mn, based on FDA parameters. It is notable that plants that grow and develop between intensive anthropogenic and severe activities are contaminated by heavy metals such as Pb, As, Mo, Co, Ni, Mn and Cr. Additionally, the concentrations of these heavy metals increase, while K, P, Fe and Zn decrease, except Se. Therefore, the consumption of plants collected in these environments can be a hazard to human health. Therefore, toxicological studies may be necessary to guarantee the safe consumption of edible plants collected in areas under intensive severe anthropogenic activities.

Overall, the estimated carcinogenic risk and total cancer risk in this pulp are represented by As, Pb and Cr, which are in higher concentrations in pulp collected in farm-margin, followed by the roadside and bush. The primary crucial heavy metal is As, presenting HQ > 1 (3.33, 2.30 and 1.34 in pulp collected in farm-margin, roadside and bush, respectively). However, quantities $\leq$ 1 g daily intake of pulp obtained in these areas can decrease the total cancer risk and are within accepted parameters and HQ < 1 for all chemical elements assessed in this pulp. This demonstrated that modern intensive agriculture farms and areas crossed by roads of large vehicle traffic are sources of pollutants that contaminate fruits and vegetables that grow in surrounding areas.

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Oxidative stability and elemental analysis of sunflower (*Helianthus annuus*) edible oil produced in Brazil using a domestic extraction machine

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The consumption of regular vegetable oils has been linked to energy acquisition, nutritional benefits, health improvement, and the regulation of metabolic diseases. This study evaluated fatty acids composition, physicochemical, thermal, oxidative, and optical properties, and quantified trace elements in the sunflower oil extracted by a domestic cold-press machine. The oil presented linoleic (54.00%) and oleic (37.29%) primary unsaturated fatty acids (91.67%), in which atherogenic (0.05), thrombogenic (0.16), hypocholesterolemic/hypercholesterolemic (21.97), peroxide (16.16), saponification (141.80), and relative density indices (0.92) demonstrated to be suitable for human consumption and possible health promotion. In addition, the concentrations of trace elements by ICP OES were ordered Zn > Fe > Al > Cu > Mn > Cr. Concentrations of Zn, Fe, Al, Cu, and Mn were lower than FAO/WHO and DRI/AI limits, while Cr concentrations exceeded the FAO/WHO limits, which can be used as an indicator of the polluted ambiance. Sunflower oil quantities daily consumption were calculated by taking into account non-carcinogenic risk (CR $< 10^{-4}$), and total non-carcinogenic hazard index (HI < 1). Based on trace elements determined in this study, the suitable quantity of

sunflower oil consumption varies according to individuals aged 8, 18, and 30 years and will be deemed 0.61, 1.46, and 1.65 g/kg, respectively, attending HI = 0.99 and CR < 10^{-4}.

KEYWORDS

cold-press, vegetable oil quality, trace elements, oil quantity consumption, non-carcinogenic indices

Introduction

One of the three main energy sources for human life activities is edible vegetable oil, whose stages (field production, harvesting, processing, storage) must be monitored (1). In addition, vegetable oils are strictly associated with health promotion and metabolic diseases prevalence such as obesity, diabetes mellitus, cardiovascular (CVD), coronary heart diseases (CHDs), systemic inflammation, atherosclerosis, cancers, etc. (2). Benefits of vegetable oils are associated with fatty acids composing them: saturated (C6:0–C20:0, no double bonds) monounsaturated (C16:1–C20:1, one double bond) and polyunsaturated (C18:2–C24:6), two up to six double bonds) (2, 3), essential components: macro- and microelements, vitamins A, C, K, and E (tocopherols, tocotrienols), phytosterols, phytostanols, carotenoids, chlorophylls, polyphenols, flavonoids, acting as antioxidants and health promoting (4–6).

Most commercially available plant seed and fruit oils are extracted using petroleum solvents, with high toxicity levels for human health (7). Moreover, their essential components are removed during refining (chemical and physical) and bleaching processes, formatting short-chain compounds [esters, polymeric triacylglycerols, trans and free fatty acids, (hydro) peroxides, dienes and trienes, and others], which act as pro-oxidants (8). These substances reduce vegetable oil shelf life and vital properties, which decrease healthy and nutraceutical properties, and are associated with the occurrence of several metabolic diseases in consumers (9) due to the higher amount of free radicals increasing oxygen species (10).

Globally, the sunflower is the second most cultivated plant after maize, and its seeds are used to treat inflammatory, hypertensive, CVD, and other diseases (11). Additionally, its oil controls tumors, diabetes, cholesterol, cancers, hypertension, hypercholesterolemia, and CHD. These benefits are associated with the synergic action of tocopherols, phytosterols, terpenoids, polyphenols, carotenoids, alkaloids, amino acids, proteins, vitamins, oleic acid, antioxidants, and other anti-inflammatory substances (4, 12, 13). Due to reducing of the production of the reactive oxygen species (ROS) and reactive nitrogen species (RNS), consequently lowering disorder metabolites [superoxide ions ($^*O_2^-$), hydroxyl (OH^-), hydrogen peroxide (H_2O_2), nitrate oxide (NO), etc.], which are linked with pro-inflammatory several metabolites [interleukin-1,−1β,−6,−8,−12 (IL-1,−1β,−6,−8,−12), Toll-like receptor 4 (TLR4), lipopolysaccharides (LPS, microbial component), hepatic nuclear factor-kappa B (NF-kB), tumor necrosis factor-alpha (TNF-α), inducible nitric oxide synthase (INOS), cyclooxygenase-2 (COX-2), lipoxygenase (LOX), cytochrome P450, nitric oxide, G protein-coupled receptor 120, etc.] potentially associated with oxidative stress and metabolic diseases due to high damage of cellular protein, lipids, and DNA (9, 14, 15).

Nevertheless, it is remarkable that food frying (pan and deep) using sunflower oil rich in linoleic fatty acid reduces its oxidation and thermal stability, in which various hazardous substances to human health form (16). To reverse this scenario, blending cold-pressed oils rich in natural antioxidants or their natural antioxidants [thymoquinone and tocopherols from black cumin (*Nigella sativa*)] with sunflower oil is highly recommended for oils stability and for consumers' health improvement (16–19).

In addition, vegetable oils are a source of macroelements such as sodium (Na), potassium (K), calcium (Ca), magnesium (Mg), phosphorus (P), and microelements such as iron (Fe), selenium (Se), manganese (Mn), chromium (Cr), zinc (Zn), aluminum (Al), barium (Ba), strontium (Sr), tin (Sn), copper (Cu), cobalt (Co), and thallium (Ti), which are essential or toxic for human health when consumed in large quantities (20, 21). In addition, some chemical elements, such as arsenic (As), cadmium (Cd) nickel (Ni), lead (Pb), mercury (Hg), and Cu are found in edible vegetable oils, which are toxic (22–24) and carcinogenic for consumers even at a low amount (25). Moreover, the presence of Ca, Co, Mg, Fe, Zn, Cu, Mn, Sn, and Ni accelerate vegetable oils' oxidation affecting their flavor, freshness, storability, and toxicity (22, 26). Therefore, cold pressing and filtration are the alternatives of the several methods to obtain healthy edible vegetable oils associated with good fatty acids and antioxidants composition, and lower amounts of hazardous chemical elements (27–29).

Interestingly, it is increasing the consumption of the unrefined edible vegetable oils, including sunflower oils obtained by cold pressed due to the high amount of natural antioxidants (tocopherols, phytosterols, carotenoids, etc.), waxes, while presenting low content of free fatty acids and phospholipids (30, 31). In addition, some studies have reported nutritional qualities

correlated with physicochemical properties, trace elements, fatty acid composition, and essential components in sunflower oil obtained by cold pressing (32). Therefore, a domestic cold-press machine is mostly recommended to obtain healthy edible vegetable oils, due to their higher amount presence of antioxidants and fatty acids, moreover, with a lower amount of heavy metals contaminants (20).

Although some studies have been carried out using different types of oils and extraction conditions, there is scarce information in the literature on the quality and mineral composition of sunflower oil extracted by a domestic cold-press machine, as well as risk assessment for human health due to the ingestion of this type of oil containing metals.

Motivated by the manuscript published by Melo et al. (20), which demonstrated that cold-extracted oils maintain their quality and chemical composition, this study aimed to evaluate for the first time the fatty acids composition, physicochemical and optical properties, thermal and oxidative stability of sunflower oil samples cold-press extracted using a domestic machine. In addition, the chemical elements Mg, Cr, Mn, Fe, Co, Ni, Cu, Zn, Cd, Al, Pb, As, and Se in sunflower oils were quantified using inductively coupled plasma optical emission spectroscopy (ICP OES) and the results were compared with DRI/AI* and FAO/WHO parameters. The fatty acid composition, physicochemical and optical properties, and thermal and oxidative qualities evaluated in this oil were compared with previous results in the literature.

Materials and methods

Sunflower seed, oil preparation, seed and oil moisture, and lipid quantification

Sunflower seeds were obtained from 10 farms in Campo Grande, Mato Grosso do Sul state, Brazil, in September 2020. Seeds were mixed and immediately dried in an air circulation oven at 40°C for 48 h. We collected crude oil from dried hull seeds using a domestic cold-press machine extractor equipped with a stainless continuous screw and drainage hole with an internal filter (Yoda Nut & Seed Cold Press Oil), Extractor-Gourmet Extractor, oil Natural, Homeup, (Yoda Europe, Cluj-Napoca, Romania). Immediately, the oil was placed into an amber and hermetic glass bottle and then used for analysis.

Moisture content was measured using milled seeds (1.0 g) subjecting in the over at 105°C for 60 min, then the sample was rested in a desiccator, until achieving constant weight. For the filtered sunflower oil (1.4 g), relative humidity was determined by the Karl Fischer technique (KEM MKC-610 Karl Fischer Moisture Titrator, Japan).

Free fatty acids (FFAs) were extracted using a Soxhlet extractor with petroleum ether as solvent at 60°C for 6 h (33).

Methylation and fatty acid profile

Fatty acid methyl esters (FAMEs) of the sunflower oil were obtained at ambient temperature. The samples (157 mg) were weighed into assay tubes, saponified with methanolic NaOH 0.5 N (4 ml), esterified with a mixture of H_2SO_4 and NH_4Cl in methanol (5 ml), then saturated with NaCl (4 ml), and finally dissolved in hexane (5 ml) (34).

FAMEs were analyzed using a gas chromatograph (model CP-3800, Varian, Santa Clara, CA, USA) equipped with a flame ionization detector, a split/splitless injector, and a stationary phase fused silica capillary column of polyethylene glycol (CARBOWAX 20 M, length 30 m × 0.25 mm, Quadrex, Santa Clara, CA, USA). Operational parameters were followed for chromatography: the injector and detector temperatures were 250°C. The column temperature was programmed to 80°C for 2 min, followed by a ramp of 4°C /min up to 220°C and kept for 13 min; hydrogen carrier gas with 1 mL min^{-1} flow and injection volume 1 μL. Retention times were compared with the respective methyl ester standards (Supelco, F.A.M.E. mix C4:0 to C24:0, Sigma-Aldrich, Darmstadt, DA, Germany) (35).

Fatty acids nutritional quality indices

The nutritional quality of sunflower oil was evaluated according to its fatty acids composition assessed by three following indices

Atherogenicity index (AI) (36).

$$AI = \frac{C12:0 + (4 \times C14:0) + C16:0}{\sum MUFA + \sum PUFA} \quad (1)$$

Thrombogenicity index (TI) (36)

$$TI = \frac{C14:0 + C16:0 + C18:0}{(0.5 \times \sum MUFA) + (0.5 \times \sum \omega 6) + (3 \times \sum \omega 3)} \quad (2)$$

Fatty acids hypocholesterolemic/hypercholesterolemic (HH) ratio (37)

$$HH = \frac{C18:1\omega 9 + C18:2\omega 6 + C20:4\omega 6 + C18:3\omega 3 + C20:5\omega 3 + C22:5\omega 3 + C22:6\omega 3}{C14:0 + C16:0} \quad (3)$$

Identity and quality characteristics of sunflower oil

Sunflower oil characterization was conducted according to the American Oil Chemist's Society (38), in triplicate, for qualification parameters: acidity (Ca 5a-40) and peroxide indices (Cd 8-53); identity parameters: relative density (Cc 10a-25), iodine-Wijis method (d 1-25), and saponification values (Cd 3-25).

Determination of oxidative stability

Sunflower oil oxidative stability was determined by the Rancimat method (873 Metrohm Co, Basel, Switzerland) by accelerated oxidation according to the European Union standardized standard EN 14112 (39). The analyses were conducted subjecting 3.0 g of oil at a constant temperature of 110°C under an airflow rate of 10 L h^{-1} through the samples, and then into a measuring vessel containing 50 ml ultrapure water Mill-Q in which the conductivity generated by volatile products during the oil decomposition was measured as a function of time.

Thermal analyses: Thermogravimetric analysis/derivative thermogravimetry, and differential scanning calorimetry

Thermogravimetric analysis (TGA)/derivative thermogravimetry (DTG) curves were obtained using TGA-Q50 (TA Instruments, New Castle, DE, USA). Samples of sunflower oil (6.0 mg) were added into a platinum pan from 10 to 550°C at a heating rate of 2°C min^{-1} under inert nitrogen and synthetic air atmosphere gases at a flow rate of 60 ml min^{-1}. In addition, DSC curves were conducted with DSC-Q20 equipment with RCS90 coupled with a cooling system (TA Instruments). The DSC curves were obtained in a calorimeter model DSC-Q20 coupled with an RCS90 refrigeration system (TA Instruments). Approximately 3.0 mg of sunflower oil was used for analysis using aluminum crucibles (Tzero standard) as support and reference, at a heating rate of 10°C min^{-1}, heating cycle followed by cooling at temperatures between −80°C and 25°C, under an inert nitrogen atmosphere with a flow rate of 60 ml min^{-1}.

Curves were obtained from TGA/DTG and DSC data, which were generated by Universal Analyzes 2000 software version 3.7A (TA Instruments).

Optical molecular analyses: UV-Visible absorption and fluorescence spectroscopy

Sunflower oil was diluted in HPLC grade hexane (spectroscopic grade 99.9%) at a concentration of 1×10^{-3} g L^{-1}. UV-Visible absorption measurements were made using a Lambda 265 UV/Vis spectrophotometer (Perkin Elmer, Waltham, MA, USA), and the spectra were collected in the 200 to 800 nm range.

Excitation-emission matrix fluorescence maps were obtained using a spectrofluorometer (FluoroMate FS-2, SCINCO). The excitation-emission maps were obtained by exciting the samples between 240 and 450 nm in steps of 5 nm and collecting the emission from 250 to 750 nm in 1 nm steps.

For the UV/Vis absorption and fluorescence measurements, the diluted sunflower oils were placed into a four-polished-sided quartz cuvette with a 10 mm optical path.

Extraction induced by emulsion breaking procedure and trace elements quantification

The extraction induced by emulsion breaking was performed according to Carneiro et al. (40) with adaptations. A falcon tube containing 3.0 ml of crude sunflower oil and 3.0 ml of ethanol was shaken for 20 s in a vertex shaker, then 3.0 ml of ultrapure water (conductivity 18.2 MΩcm, Millipore, Biocel, Germany), 0.76 ml of Triton x-100, and 3.0 ml of HNO_3 were added, and then mixed with a vortex mixer for 20 min.

The concentration of Al, Cr, Cu, Fe, Mn, and Zn in sunflower oil samples was obtained using an ICP OES with an Axial Plasma (iCAP 6000 Series, Thermo Scientific, Cambridge, UK). Standard solutions were prepared by diluting a standard multi-element stock solution (SpecSol, Quinlab, Jacarei, SP, Brazil) containing 1,000 mg L^{-1} of each element. Nine concentrations were used to build calibration curves for the quantitative analyses of oil. The concentration for the elements was 0.01–5.0 mg L^{-1} range. The setup of ICP OES instrumental conditions for elemental analyses was the same used by Melo et al. (20). Table 1 summarizes the operational condition used in the ICP OES apparatus analysis as wavelength, limit of detection (LOD), limit of quantification (LOQ), and correlation coefficient (R^2) in the current study.

One blank and nine calibration curves were generated using the following concentrations: 0.005, 0.01, 0.025, 0.05, 0.1, 0.25, 0.5, 1.0, and 2.0 mg kg^{-1} of each element standard.

An addition/recovery test for the elements under study was carried out in a sunflower oil sample by spiking 0.5 mg L^{-1} of each analyte. The method had a recovery interval of 80–110%

TABLE 1 The ICP OES operating conditions for analysis.

Elements	Wavelength (nm)	LOD (mg/kg)	LOQ (mg/Kg)	Correlation (R^2)
Al	396.152	0.0114	0.0380	0.9995
As	193.696	0.0048	0.0159	0.9996
Cd	228.802	0.0007	0.0024	0.9994
Co	238.892	0.0016	0.0054	0.9995
Cr	425.435	0.0015	0.0049	0.9994
Cu	327.396	0.0014	0.0048	0.9995
Fe	259.940	0.0193	0.0644	0.9991
Mg	285.213	0.0675	0.2251	0.9984
Mn	257.610	0.0004	0.0012	0.9996
Ni	221.647	0.0023	0.0077	0.9994
Pb	220.353	0.0070	0.0233	0.9996
Se	196.026	0.0080	0.0267	0.9997
Zn	213.856	0.0018	0.0060	0.9991

for the spike of 0.5 mg L^{-1}, to the established limit proposed by the Association of Official Analytical Chemists (41).

Human health risk assessment

The concentration of the chemical elements in sunflower oil was compared with the FAO/WHO recommended intake standards and hazards quotient. The non-carcinogenic was calculated according to the equation adopted by Machate et al. (42). Cancer risk is the probability of an individual developing any cancer type over a lifetime due to a specific exposure to a hazardous mineral. The chronic daily intake dose (CDI) of carcinogenic elements (mg/kg/day) and slope factor (SF) of Cr is 0.5 mg/kg/day, according to Equation (4).

$$\text{Cancer Risk} = \text{CDI} \times \text{SF} \tag{4}$$

Cancer risk is a sum of individual chemical elements in different exposure pathways to develop cancer in a person, which is the total cancer risk (CR). According to US UPA (43), acceptable values of cancer risk range from 10^{-6} to 10^{-4}, while values $> 10^{-4}$ are considered inadmissible.

The human health risk of trace element consumption dose was calculated on the chronic daily intake dose (CDI, mg/kg/day) for a chemical contaminant element in the sunflower oil intake quantity CDI calculated according to Equation (5).

$$\text{CDI}_{\text{oil}} = \frac{\text{C} \times \text{IR} \times \text{EF} \times \text{ED}}{\text{BW} \times \text{AT}} \tag{5}$$

where CDI_{oil} – chronic daily oil intake dose; C – chemical element concentration in the crude sunflower oil sample (mg kg^{-1}); IR – ingestion rate g/day; EF – exposure frequency (365 days/year); ED – exposure duration; BW – body weight (kg), estimated 26, 62, and 70 kg for 8, 18, and 30 years old, respectively. AT – average time (ED × 365 days/year).

The risk to human health by the intake of trace element contaminated food was estimated using a hazard quotient (HQ), which is a ratio of CDI and chronic oral reference dose (RfD), determined by the following Equation (6):

$$\text{HQ} = \frac{\text{CDI}}{\text{RfD}} \tag{6}$$

The RfD values were previously established by the Joint Food and Agriculture Organization/World Health Organization Expert Committee on Food Additive (44) and the United States Environmental Protection Agency (45). The RfD (mg/kg/day) values are: Al = 1.0; Cr = 0.003; Cu = 0.04; Fe = 0.7; Mn = 0.14; and Zn = 0.3 (46). As shown in Equation (6), hazard quotient toxic risk on each trace element and their sum, Equation (7) (total non-carcinogenic hazard index) HI < 1, safe food consumption, while HI > 1, health risk food consumption.

$$\text{HI} = \text{HQ}_{\text{Al}} + \text{HQ}_{\text{Cr}} + \text{HQ}_{\text{Cu}} + \text{HQ}_{\text{Fe}} + \text{HQ}_{\text{Mn}} + \text{HQ}_{\text{Zn}} \tag{7}$$

Results and discussion

Oil preparation, sunflower seed, oil moisture, and lipid quantity

Sunflower seed yielded 260 mg g^{-1} (26%) of oil, lower than industrially obtained using petroleum solvents (36–50%) (47). However, oil extracted by cold-press yielded a higher amount of beneficial nutritional components such as tocopherols, phytosterols, terpenoids, phenolic acids, carotenoids, chlorophylls, antioxidants, waxes (C36–C48),

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TABLE 2 Fatty acid composition of sunflower oil extracted by cold pressing.

Fatty acids	Mean ± Standard deviation (%)
Myristic (C14:0)	0.03 ± 0.00
Palmitic (C16:0)	4.13 ± 0.05
Palmitoleic (C16:1)	0.04 ± 0.00
Margaric (C17:0)	0.04 ± 0.00
Heptadecenoic (C17:1)	0.02 ± 0.00
Stearic (C18:0)	3.13 ± 0.08
Oleic (C18:1n9c)	37.29 ± 0.24
Linoleic (C18:2n6c)	54.00 ± 0.39
α-Linolenic (C18:3n3c)	0.05 ± 0.00
Araquidic (C20:0)	0.20 ± 0.00
Gondoic (C20:1)	0.13 ± 0.00
Eicosapentanoic (C20:5n3c)	0.06 ± 0.00
Behenic (C22:0)	0.53 ± 0.01
Docosadienoic (C22:2)	0.08 ± 0.00
Tricosylic (C23:0)	0.08 ± 0.00
Lignoceric (C24:0)	0.17 ± 0.01
Σ SFAs	8.33
Σ MUFAs	37.48
Σ PUFAs	54.19
Σ UFAs	91.67
Total FAs	100
Atherogenic index	0.05
Thrombogenic index	0.16
Hypocholesterolemic/hypercholesterolemic	21.97

Σ SFAs, sum of saturated fatty acids; Σ MUFAs, sum of monounsaturated fatty acids; Σ PUFAs, sum of polyunsaturated fatty acids; FAs, fatty acids; ND, non-detectable; defined as < 0.05%.

oleic and linoleic fatty acids, phospholipids, unsaponifiable matters, and others; in contrast, the hazardous components (trace metals) were extracted in lower amounts (29, 48, 49). The cold-pressing technique is widely recommended because it provides oils with healthy components, including their by-products (oil cake) (32), of which adequate consumption is correlated with regulating metabolic diseases (12).

The moisture determination in sunflower seeds yielded 72.76 mg g^{-1} (7.28%) of dehydrated water, whereas dry matter was 927.24 mg g^{-1} (92.72%), better for fatty acid and essential components integrity, which are relevant for nutrition functionality (50). Sunflower oil moisture was 998 μg, (0.07%) of evaporated water, demonstrating the importance of this oil in food system quality, due to less susceptibility to oxidative stress and lipid oxidation by the action of pro-oxidants substances (51). Refined flour of sunflower seed (1.0 g) yielded 493.82 mg g^{-1} (49.38%) of FFAs, demonstrating its relevance for nutritional value and a good source of vegetable oil (29).

TABLE 3 Physicochemical characteristics of sunflower oil compared with Codex Alimentarius parameters.

Parameters	Sunflower oil	Maximum values
Peroxide index (mEq/kg)	16.61 ± 0.2	≤20 (55) (a)
Iodine index (g I_2/100 g)	131.53 ± 0.1	118–141 (56) (b)
Acidity index (mg KOH/g)	0.54 ± 0.0	4.0 (56) (a)
Saponification index (mg KOH/g)	141.80 ± 1.92	184–196 (56) (b)
Relative density (20°C)	0.92 ± 0.1	0.92–0.93 (56) (c)

(a) Parameter for cold-pressed and virgin oils; (b) parameters for refined sunflower oils; (c) parameter for crude sunflower oil.

Fatty acid profile

Table 2 presents the composition and quantity of fatty acids yielded in this study, with profiles in decreased order: linoleic (54.00%) > oleic (37.29%) > palmitic (4.13%) > stearic (3.17%) > behenic (0.55%) > araquidic (0.20%) > lignoceric (0.17%) > gondoic (0.13%) > docosadienoic = tricosylic (0.08%) > eicosapentanoic (0.06) > α-linolenic (0.05%) > palmitoleic = margaric (0.04%) > meristic (0.03%). In this study, most quantified fatty acids, linoleic/oleic ratios were proportionally shown at 1.44:1, better than oils extracted by n-hexane as solvent 3.24:1 (32, 52).

According to our results, the sunflower oil extracted using a domestic cold press machine yielded a higher amount of oleic acid and the highest hypocholesterolemic (HH) ratio (Table 2) compared with one that applied n-hexane (oleic, 19.81%; linoleic, 64.35%; HH, 0.16) (32), massively used by industry to obtain edible vegetable oils.

Therefore, long-term diets of vegetable oils rich in linoleic acid and the lowest hypocholesterolemic index are associated with the prevalence and incidence of several metabolic diseases (diabetes mellitus, coronary and inflammatory diseases, cancer, and obesity) (4, 6, 53). Moreover, in the human body, linoleic acid is converted into arachidonic acid (n-6 PUFAs), which is the pro-inflammatory precursor of prostaglandin and leukotriene synthesis at the cyclooxygenase (COX-2), lipoxygenase (LOX), and cytokines (TNF-α, IL-1, IL-1β, IL-6, IL-8, IL-12, NF-kB, NO, LTs cytochrome P450, protein-coupled receptor 120), which compete with n-3 PUFAs enzymes during the biosynthesis pathway of long- (LC-PUFAs) and very-long-chain fatty acids (VLC-FAs) associated with anti-inflammatory effects (9, 14, 15).

Fatty acid nutritional quality indices and characteristics of sunflower oil

Table 2 depicts nutritional quality indices: atherogenicity index (AI), thrombogenicity index (TI), and hypocholesterolemic/hypercholesterolemic (HH)

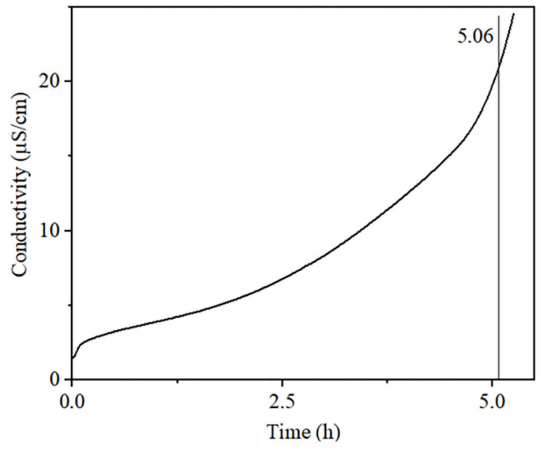

FIGURE 1
Conductivity vs. time determined by the Rancimat method. Oxidation stability of sunflower oil conducted at 110°C with an airflow of 10 L h^{-1}.

ratio calculated as 0.05, 0.16, and 21.97, respectively. Sunflower oil obtained by the cold-press machine presents better values associated with regulating several metabolic diseases for consumers than another extracted by petroleum solvent (54).

Table 3 presents physicochemical profiles revealing that this sunflower oil is suitable for human consumption, and its averages can be associated with oxidative stability, authenticity, quality, and identity (57). The iodine index (131.53) was found between the Codex Alimentarius parameters, which correspond to unsaturated fatty acids (91.67%). Moreover, this oil is characterized by the lowest acidity and peroxide indices, demonstrating its lipid stability against rancidity due to the lowest autoxidation products (ketones, aldehydes, hydroxyl alkenals, and dienes) formation which became off-flavor and toxic food for consumers associated with pro-oxidants action (processing manner, oxygen, heat, light, and metals) (9, 58). In addition, this oil presented a lower saponification index (141.80 mg KOH/g oil) compared with parameters and others obtained using petroleum solvent (188 and 189 mg KOH/g oil) (59), represented by the long-chain fatty acids (palmitic, stearic, oleic and linoleic acids), which can be used to identify these oils.

Relative density is the relevant parameter correlated with edible vegetable oil absorption and mass transfer rates during cooling or melting, better to lower values than the parameters (60). Sunflower oil obtained using a domestic machine presented a lower average (0.91) compared with that extracted using solvents (0.92–0.96) (61, 62). Although, these values represent a small difference among them, however, other parameters above referred can be used to identify the authenticity and origin of these oils.

Determination of oxidative stability

Rancimat data revealed that the crude sunflower oil (unsaturation 91.67%) presented an induction period (IP) of 5.06 h (Figure 1). This behavior can be attributed to natural antioxidants in this oil as well observed to refined sunflower oil (unsaturation 88.40%), which the IP shifted from 5.5 to 7.5 h, respectively, in control and sunflower oil added polyphenol, subjected under a temperature of 110°C with airflow 20 L h^{-1} (63). Furthermore, the unsaturation amount of fatty acids is another relevant characteristic that is inversely proportional to oxidative stability. Sunflower hybrid oils (H) which presented higher iodine value (IV) showed lower IP, for instance, e.g., H19: (IV = 127, IP = 3.32 h) has lower IP compared with H21 (IV = 81, IP = 9.55 h) (64). Moreover, among unsaturation fatty acids, cold-pressed sunflower oil (SO) that presented a higher amount of oleic than linoleic fatty acids was more oxidative stably H20 (55), as well as SO1 (oleic, 86.52% and linoleic, 5.49%, IP = 19.87 h), while SO2 (oleic, 18.52% and linoleic, 66.02%, IP = 6.42 h) (65).

Therefore, to increase the stability of sunflower oil (rich in linoleic acid), it is recommended to blend it with oil rich in stable antioxidants such as tocopherols and thymoquinone or apply their natural antioxidants (17, 18).

Thermogravimetric analysis/derivative thermogravimetriy, and differential scanning calorimetry

Thermogravimetric analysis/derivative thermogravimetry curves of crude sunflower oil in the presence of synthetic air and nitrogen atmospheres are shown in Figure 2. The TGA/DTG curves of sunflower oil submitted under a synthetic air atmosphere show three steps of thermal decomposition that are illustrated in Figure 2A and summarized in Table 4. The first step can be attributed to moisture linked by natural antioxidants (polyphenols, carotenoids, stanols, and vitamins A and C), dimers, trimmers, polymers, PUFAs, and other compounds formed from PUFAs (54.19%), represented by linoleic acid (54.00%). In addition, this process can be influenced by Zn (6,6228 mg/kg) and Fe (1.6637 mg/kg), both metals that negatively influence on oxidation stability (22, 26). The first stage of thermal stability is the most important, as it demonstrates that this cold-pressed sunflower oil can be heated up to 125°C without undergoing oxidative degradation.

The second step of mass loss is attributed to products formed in the first step plus MUFAs (37.48%) decomposition represented by oleic fatty acid (37.29%), whose double bonds of MUFAs are broken and become SFAs of the oil. The third step of mass decomposition is attributed to the above fatty acids plus SFAs (7.26%) (palmitic (4.13%), stearic acids (3.13%), and

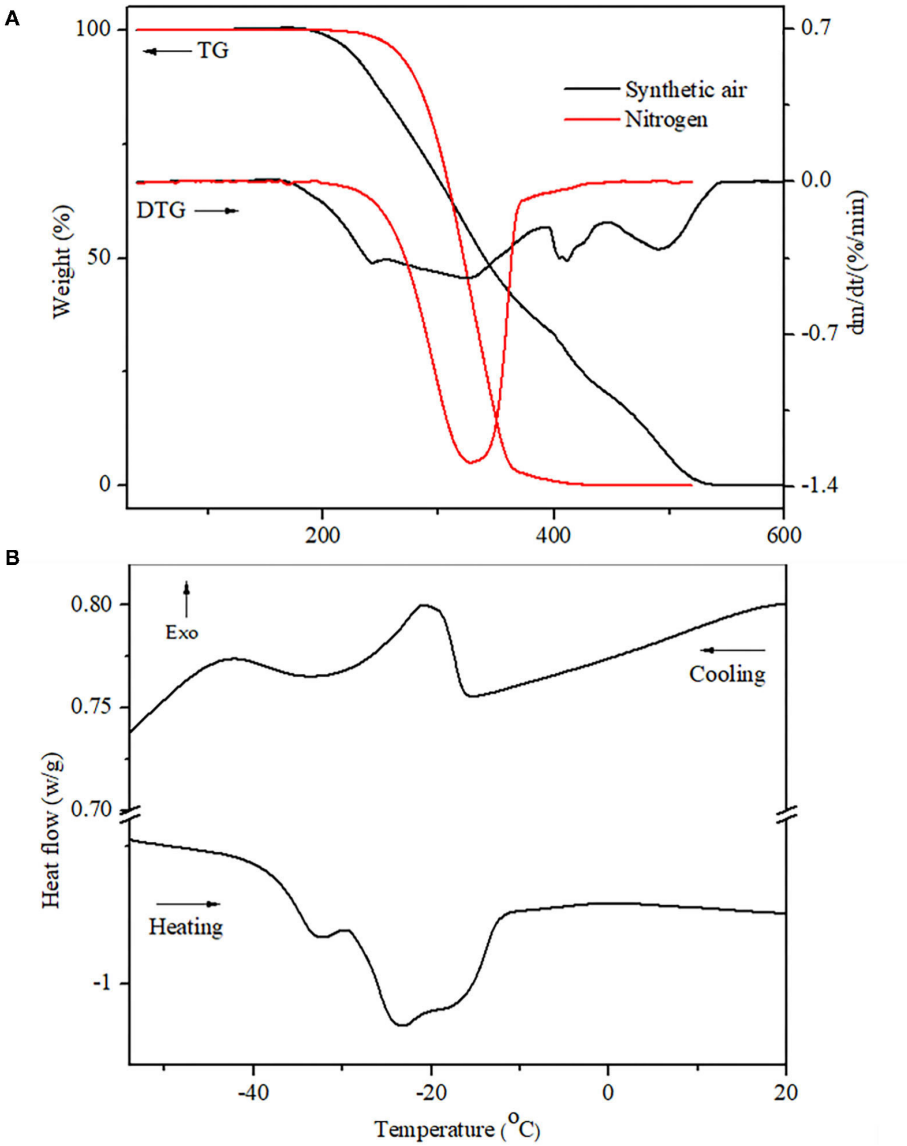

FIGURE 2
Thermal analyses of the sunflower oil. **(A)** TGA/DTG curves at 2°C min^{-1} heating from 10 to 550°C, under synthetic air and nitrogen atmospheres flow at 60 ml min^{-1} in dynamic conditions; **(B)** DSC curves of cooling and heating under the nitrogen atmosphere.

waxes (C36–C48), of which the majority are represented by C36, C37, C40, and C41, as previously reported in cold-pressed sunflower oil (49). The minor last mass loss is attributed to carbonaceous residue substances. This behavior was reported in commercial sunflower oil, although with more thermal stability compared with the current study (66–68). Moreover, cold press and unrefined oil present more oleic than refined one (10), natural antioxidants (69) and minerals, which are healthy and regulators of several metabolic diseases (9, 68, 70), and economic and ecological benefits from food by-products (71) than refined ones. Moreover, synthetic antioxidants, butylated hydroxyanisole (BHA), butylated hydroxytoluene (BHT), tert-butylhydroquinone (TBHQ), and others (10, 72) replace natural antioxidants in industrial edible vegetable oils, which are associated with the prevalence of metabolic diseases (cellular damage, cancers) and environmental contamination (73, 74).

In the nitrogen atmosphere, the oil showed one high step mass decomposition justified by a higher amount of unsaturated fatty acids (oleic and linoleic fatty acids) compared with SFAs. The minor last mass loss is attributed to formed carbonaceous residual substances.

In Figure 2B, DSC curves show two exothermic crystallization peaks, which the first attributed to SFAs and MUFAs, with T_{onset} at −16.57°C and enthalpy peak at 1.979 J/g, followed by a second peak representing PUFAs observed at

TABLE 4 TGA/DTG curves of sunflower oil obtained under synthetic air and nitrogen atmospheres.

Sample	Atmosphere	Steps	Temperature range (°C)		Event mass loss (%)	Residual mass (%)
			Initial	Final		
Sunflower oil	Synthetic air	1st	126.16	254.34	15.48	0.21
		2nd	254.34	392.17	49.74	
		3rd	254.34	447.18	14.57	
	Nitrogen	1st	236.19	465.90	99.09	0.33

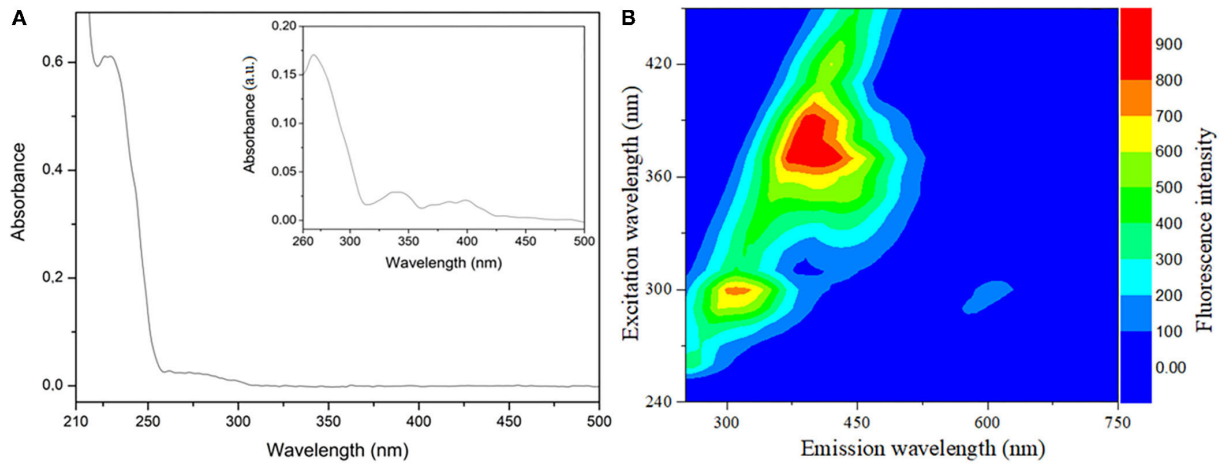

FIGURE 3
Optical molecular analysis used sunflower oil diluted in hexane HPLC 99.9% at 1 × 10^{-3} g L^{-1}. **(A)** UV-Visible absorption spectrum wavelength collected between 200 and 800 nm and the inset "zoom"; **(B)** Emission-excitation fluorescence spectrum map with excitation obtained between 240 and 450 nm and emission (250–750 nm).

−34.52°C, with enthalpy peak at 1.466 J/g. Moreover, heating the sunflower oil, two peaks were also observed, the first peak corresponding to MUFAs, whose T_{onset} was observed at −36.27°C and enthalpy at 1.866 J/g. The T_{onset} of the second peak appeared at −28.07°C and the enthalpy peak at 24.59 J/g.

In the current study, DSC results were observed in lower temperatures than commercial sunflower oil due to their fatty acids and natural antioxidant composition, which influence its thermal oxidative levels (75). The TGA and DSC analyses are used to qualify, authenticate, and recognize vegetable oils, thus avoiding their adulteration, falsification, and consumption of improper products.

Optical molecular analyses: UV-Visible absorption and fluorescence spectroscopy

Figure 3A illustrates sunflower oil UV-Vis absorption spectrum with two major absorbance regions one in the 223–236 nm range and the other from 257 to 452 nm (inset of Figure 3A). The first absorption band can be attributed to phytocholesterols (phytostanols), phytosterols, and tocopherols (vitamin E). The second region corresponds to carotenoids and fatty acids (linoleic, oleic acids) (6, 76). The consumption of oils rich in phytocholesterols, carotenoids, tocopherols and phytosterols, polyphenols, and unsaturated fatty acids are widely correlated with avoiding and regulating several metabolic diseases (3, 9).

The UV-Vis is used to monitor oxidation, identity, and authenticity, as the absorption in the 400–520 nm range appears higher in adulterated sunflower oil (77).

Figure 3B shows the excitation–emission map of sunflower oil exhibiting two intense bands. The first region presents emissions in the 297–327 nm range when excited between 290 and 310 nm. The second region of emission is observed between 360 and 445 nm due to excitation in the 360–400 nm range. These fluorescence bands can be correlated with the presence of vitamin E (tocopherols and tocotrienols), carotenoids, chlorophyll, and unsaturated fatty acids in sunflower oil (78).

The fluorescence emission wavelength ranging from 400 to 500 is used to identify, qualify, and authenticate original and adulterated vegetable edible oils due to the oxidation of fatty acids products and tocopherols (79).

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TABLE 5 Trace elements in sunflower oil quantified by ICP OES (mg/kg ± SD) compared with nutritional recommendations for adult, pregnancy, lactation, and children by RDA/AI and FAO/WHO.

Trace elements	Concentration (mg/kg)	FAO/WHO (mg/kg)	Dietary reference intakes (DRI) and adequate intake (AI*) (mg/day) (80)								
			Children	Males		Females		Pregnancy		Lactation	
			8 y. old	18 y. old	30 y. old	18 y. old	30 y. old	18 y. old	30 y. old	18 y. old	30 y. old
Cr	0.0242 ± 0.0049	0.002 (81)	0.015*	0.035*	0.035*	0.024*	0.025*	0.029*	0.030*	0.044*	0.045*
Mn	0.2372 ± 0.0051	3.00 (82)	1.5*	2.2*	2.3*	1.6*	1.8	2.0*	2.0*	2.6*	2.6*
Fe	1.6637 ± 0.0393	14.00 (82)	10	11	8	15	18	27	27	10	9
Cu	0.2791 ± 0.0094	0.90 (82)	0.44	0.89	0.9	0.89	0.9	1	1	1.3	1.3
Zn	6.6228 ± 0.0788	15.00 (82)	5	11	11	9	8	12	11	13	12
Al	1.0401 ± 0.0199	5.00 (83)	ND	ND	ND	ND	ND	ND	ND	ND	ND

Elements Mg, Co, Ni, Cd, Pb, As, and Se < LOD; NE, not established; ND, not determined; *The value for AI is used when there are no calculated values for the RDA.

Trace elements concentration in sunflower oil

Table 5 summarizes the data of six trace elements quantified in sunflower oil compared with Codex Alimentarius contents for oils and dietary reference intakes (DRIs).

Beyond the endogenous biological processes, plants acquire minerals from the soil, besides exogenous processes (environment pollution by industries, transports, mechanization, fertilizers, and pesticides used in agriculture), which influence raw material for edible plant production (42). Moreover, minerals can contaminate edible vegetable oils during production, refining, and storing processes.

Minerals (Ag, As, Be, Ca, Cu, Zn, Fe, Mg, Mn, Cd, Co, Na, K, Ni, Pb, and V) are used to monitor and qualify edible vegetable oils: corn (*Zea mays*), hazelnut (*Corylus avellana*), olive (*Olea europaea*), and sunflower in Turkey (84), pequi (*Caryocar brasiliense*), primrose (*Oenothera biennis*), avocado (*Persea americana*), coconut (*Cocos nucifera*), grape seed (*Vitis vinifera*), babassu (*Attalea speciosa*), and licuri (*Syagrus coronata*) in Brazil (40), and several varieties in China (26) and Iran (24).

Mineral concentrations can be used as a relevant fingerprint to distinguish the provenance, originality, and adulterated edible vegetable oils, promoting health benefits. The average concentrations of trace elements quantified in sunflower oil in decreased order are of Zn (6.6228 mg/kg) > Fe (1.6637 mg/kg) > Al (1.0401 mg/kg) > Cu (0.2791 mg/kg) > Mn (0.2372 mg/kg) > Cr (0.0242 mg/kg). Only Cr concentrations exceeded 1,210% of FAO/WHO limits, which can be influenced by the vehicle fumes, fertilizers, and pesticides used, as reported in the intensive agriculture modern farming, as well as the cultivated areas located near the road with high vehicle traffic (42, 85). In contrast, Zn, Fe, Al, Cu, and Mn concentrations were lower than FAO/WHO and DRI/AI* limits. Moreover, Cr and Zn concentrations were within DRI/AI* limits, while Cu, Fe, and Mn concentrations were quantified lower than DRI/AI* limits (Table 5).

Furthermore, Al concentration was higher than quantified in industrial refined oils of avocado, primrose, babassu, licuri, pequi, olive, and grape seeds (0.04–0.52 mg kg^{-1}) (32). In contrast, Zn, Cu, and Mn amounts were reported between those found in commercially refined oils of sunflower, olive, canola, soybean, corn, and hazelnut corresponding to 1.03–9.54, 0.05–4.504, and 0.04–1.76 mg kg^{-1}, respectively. The concentrations of Cr and Fe were lower than reported in sunflower, olive, canola, soybean, corn, and hazelnut oils corresponding to 0.0126–7.106 and 7.78–28.93 mg kg^{-1}, respectively (24, 84, 86).

Thus, in light of the acceptable concentrations of trace elements regarding referential parameters, the consumption of the sunflower oil herein studied can be beneficial, because Cr, Cu, Fe, Mn, and Zn play essential physiological roles in defense response, protein construction, enzymatic reactions, signaling pathways, regulation of oxidative stress and metabolic diseases, and others (87). However, lower Al concentrations are associated with reduced occurrence of metabolic diseases and dysfunctions, such as cancers, Alzheimer's and Parkinson's diseases, inhibited enzymatic, cytotoxic and neurotoxic reactions, gut imbalance, skeletal disorders, and others (42).

Health risk assessment

Most studies reported daily consumption of vegetable oils from 25 to 30 g kg^{-1} (26, 88). Other studies demonstrated that quantities of cooking oils depend on the type of dishes: pure vegetables range from 9 to 167 g kg^{-1}, with a mean of 56 g kg^{-1}, pure meat from 4 to 353 g kg^{-1} (142 g kg^{-1}), and mixed meat-vegetable 7 to 394 g kg^{-1} (110 g kg^{-1}) (89).

However, the calculated quantities of the sunflower oil daily intake (g kg^{-1}) in comparison with other studies based on the concentration of trace elements regarding acceptable

TABLE 6 Non-carcinogenic risk (CR), hazard quotient (HQ), and total non-carcinogenic hazard index (HI) of trace elements on ingestion rate (IR g/kg) of sunflower oil obtained by cold-press (Brazil and Romania) and commercially available (China and Turkey).

Study	Years old	IR (g/day)	Index	Trace elements														HI
				Co	Cr	Cu	Fe	Mn	Ni	Pb	Se	Zn	Li	Mo	Cd	As	Al	
Brazil (Current study)	8	0.61	CR	-	2.8×10^{-4}	-	-	-	-	-	-	-	-	-	-	-	-	
			HQ	0	0.18926	0.1637	0.05576	0.03975	0	0	0	0.51794	-	-	0	0	0.0244	0.99
	18	1.46	CR	-	2.9×10^{-4}	-	-	-	-	-	-	-	-	-	-	-	-	
			HQ	0	0.18996	0.16431	0.05597	0.0399	0	0	0	0.51985	-	-	0	0	0.02449	0.99
	30	1.65	CR	-	2.9×10^{-4}	-	-	-	-	-	-	-	-	-	-	-	-	
			HQ	0	0.19014	0.16447	0.05602	0.03994	0	0	0	0.52036	-	-	0	0	0.02452	0.99
Romania (32)	8	0.037	CR	-	3.6×10^{-4}	-	-	-	-	-	-	-	-	-	-	-	-	
			HQ	-	0.2372	0.007	0.00002	0.00793	0.01352	-	0.333	0.00043	0.14231	0.25615	-	-	-	0.99
	18	0.08	CR	-	3.2×10^{-4}	-	-	-	-	-	-	-	-	-	-	-	-	
			HQ	-	0.21505	0.00677	0.00002	0.00719	0.01226	-	0.30194	0.00039	0.12903	0.23226	-	-	-	0.99
	30	0.099	CR	-	3.5×10^{-4}	-	-	-	-	-	-	-	-	-	-	-	-	
			HQ	-	0.23571	0.00743	0.00002	0.00788	0.01344	-	0.33094	0.00042	0.14143	0.25457	-	-	-	0.99
China (26)	8	0.419	CR	-	-	-	-	-	-	1.4×10^{-6}	-	-	-	-	2.1×10^{-5}	2.7×10^{-5}	-	-
			HQ	-	-	0.02659	0.67224	0.05122	0.0274	0.04029	-	0.06607	-	-	0.056565	0.05909		0.99
	18	0.99	CR	-	-	-	-	-	-	1.4×10^{-6}	-	-	-	-	2.1×10^{-5}	2.6×10^{-5}	-	
			HQ	-	-	0.02635	0.66608	0.05075	0.02715	0.03992	-	0.06547	-	-	0.05605	0.05855	-	0.99
	30	1.128	CR	-	-					1.4×10^{-6}	-	-	-	-	2.1×10^{-5}	2.7×10^{-5}	-	
			HQ	-	-	0.02659	0.67220	0.05122	0.02739	0.04029	-		-	-	0.05656	0.05909	-	0.99
Turkey (84)	8	0.0093	CR	-	-	-	-	-	-	3×10^{-5}	-	-	-	-	5.1×10^{-7}	-	-	
			HQ	0.0065	-	0.00099	0.05435	0.03097	-	0.90323	-	0.00132	-	-	0.00134	-	-	0.99
	18	0.0224	CR	-	-	-	-	-	-	3.1×10^{-5}	-	-	-	-	5.2×10^{-7}	-	-	
			HQ	0.10074	-	0.01539	0.8419	0.0048	-	0.01399	-	0.00133	-	-	0.000000021	-	-	0.99
	30	0.0253	CR	-	-	-	-	-	-	3.1×10^{-5}	-	-	-	-	5.2×10^{-7}	-	-	
			HQ	0.00651	-	0.0099	0.05437	0.03098	-	0.90357	-	0.02043	-	-	0.001358971	-	-	0.99
Turkey (86)	8	0.0097	CR	-	1.3×10^{-3}	-	-	-	-	3.2×10^{-7}	-	-	-	-	8.1×10^{-6}	-	-	
			HQ	-	0.88369	0.02766	0.00475	-	0.03231	0.00933	-	0.0079	-	-	0.02127	-	-	0.99
	18	0.0234	CR	-	1.3×10^{-3}	-	-	-	-	3.2×10^{-7}	-	-	-	-	8.2×10^{-6}	-	-	
			HQ	-	0.89398	0.02799	0.00481	-	0.03268	0.00944	-	0.00799	-	-	0.02151	-	-	0.99
	30	0.0264	CR	-	1.3×10^{-3}	-	-	-	-	3.2×10^{-7}	-	-	-	-	8.2×10^{-6}	-	-	
			HQ	-	0.89333	0.02797	0.00481	-	0.03266	0.00943	-	0.00799	-	-	0.0215	-	-	0.99

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non-carcinogenic risk (CR) $< 10^{-6}$ to 10^{-4} and total non-carcinogenic hazard index (HI) < 1 are summarized in Table 6. Based on the country of origin, it is remarkable that the quantity of sunflower oil daily intake is independent of the obtained by cold-pressed (Brazil and Romania) or petroleum solvent extraction (China and Turkey). Regarding trace elements concentrations in sunflower oil, the quantity of sunflower oil daily intake by individuals aged 8, 18, and 30 years old are, respectively, described in decreased order for Brazil (0.61, 1.46, 1.65 g/day) > China (0.41, 0.99, 1.12 g/day) > Romania (0.037, 0.08, 0.099 g/day) > Turkey (0.0097, 0.0234, 0.0264 g/kg) > Turkey (0.0093, 0.0224, 0.0253 g/kg).

Thus, given the findings of the current study (mineral concentrations), it seems relevant to explore the calculating amount of vegetable oil consumption per day based on trace elements quantified from different origins to be used for health promotion and regulation of several metabolic diseases.

Conclusion

The assessed sunflower oil obtained by a domestic cold-pressing demonstrates optimal qualitative properties for consumption, correlated with observed results on fatty acids composition, physicochemical optical features, thermal and oxidative qualities, and trace elements compared with DRI/AI* and FAO/WHO parameters herein evaluated. However, Cr concentration in sunflower oil was above FAO/WHO limits, which can be used as an indicator of ambient pollution.

However, to obtain values of HI < 1, and CR $< 10^{-4}$, the maximum sunflower oil daily consumption varies between 0.61, 1.46, and 1.65 g kg^{-1}, respectively, for individuals aged 8, 18, and 18 years. Moreover, the calculated results based on trace elements concentration regarding HI < 1 and CR $< 10^{-4}$ indices of the sunflower oil previously qualitative approved show lower daily intake compared with prior daily consumption varying from 25 to 142 g kg^{-1}.

Thus, it is expected that this quantitative daily consumption of sunflower oil here presented can be used for other vegetable oils and several foodstuffs for health improvement and metabolic disease regulation.

Data availability statement

The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation.

Author contributions

DM: conceptualization of the topic, methodology, investigation, formal analysis, writing the original draft, visualization, and data curation. EM, FM, LO, and AC: methodology. DB, AP, LC, KF, PH, MV, RO, and RG: investigation and formal analysis. VN and DM: writing, reviewing, and editing. VN: supervision, funding acquisition, and project administration. All authors have read and approved the final version of the manuscript, ensure the accuracy and integrity of the work, and agree to be accountable for all appearances.

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Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Publisher's note

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