First Determination of Mineral Composition of the Leaf Chicory (Cichorium Intybus L.) Used in Human Nourishment in the Midwest of Brazil and Comparison with Dietary Reference Intakes for Children and Adults

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Abstract

Introduction: Radicchio (Cichorium intybus L.) is a leaf chicory and is grown as a leaf vegetable which usually has white-veined red or purple leaves and it belongs to the Asteraceae family. In several countries is consumed mainly as salad, but no studies on their elemental composition has been done, principally in Brazil.

Objective: The aim of present work was to measure the macroelements (Na, K, Ca, Mg and P) and microelements (Cr, Cu, Fe, Mn, Mo, Zn, Al, Cd, Ni, Co and Si) in the leaf Chicory used as human nourishment in the Campo Grande, State of Mato Grosso do Sul, Brazil.

Method: Chemical digests of samples were prepared using HNO₃ and H₂O₂ and then placed in the microwave digestion system. After digestion, the concentrations of the elements in leaf chicory were determined by the technique of Inductively Coupled Plasma - Optical Emission Spectrometer (ICP-OES, Thermo Scientific - iCAP 6000 Series). Results obtained of the concentrations of leaf chicory were compared with the dietary reference intakes.

Results: Detected concentration of macroelements in leaf chicory decreases in the order: K > P > Ca > Mg > Na. As well as the con-
Introduction

The nutrition is one of the main factors of concern and are a major cause of mortality in old age [1]. According the World Health Organization (WHO), the malnutrition contribute to more than one third of all child deaths. Due to rising food prices, lack of access to highly nutritious foods is a common cause of malnutrition. There are others factors that contribute to malnutrition such as inadequate breastfeeding and the wrong foods [2]. The WHO estimate in 2013 was that 5.2 million deaths worldwide would be attributable to inadequate consumption of fruits and vegetables [3]. At present, only a small minority of the world’s population consumes the average intake of fruits and vegetables recommended by countries or health services. In fact, studies have found that regular intake of fruits and vegetables reduces the risk of cardiovascular diseases, cancers, diabetes and hypertension [4].

In fact, vegetables can be healthy alternatives to human food. Vegetables are important sources of many nutrients and are recommended for supplementation of diet in human. In this context, Brazil has great number of vegetables and wide variety of fruits that is not yet well investigated [5]. Vegetables as *Cichorium intybus* Linnaeus (leaf chicory) are important foods in the diet of the Brazilian people as well as in others countries [6]. Chicory is a traditional European horticultural crop [7]. In Brazil and Italy, the leaf chicory is called “radicchio”, being consumed mainly as salad. Radicchio production has increased in Brazil, where it is currently considered an important vegetable due to high proportion of the agricultural income. Radicchio is a perennial, and has a quick growing with red-colored leafy (Figure 2) [8]. In recent work [6], the botanical classification the genus *Cichorium* is reported and a detailed description of the most important cultivated biotypes is presented.

In Brazilian folk medicine, some species of chicory aids digestion and colon cleansing by its fiber content. In addition, chicory has been used to fight concentration of microelements decreases in the order: Fe > Al > Si > Mn > Zn > Cu > Cr > Ni > Cd > Mo > Co. Leaf chicory is excellent source of K, Ca, Mg, P, Cr, Cu, Fe, Mn, Mo and Zn for children and adults. On the other hand, leaf chicory is not considered a source of sodium for children and adults. Concentration of K, Cr, Fe, and Al are above the limit recommended limit by FAO/WHO (1984) and others countries.

Conclusions: Leaf chicory has macro and microelements in ample amount; it can be used as an important part of people’s diets. Since it not exceed allowable limits set by WHO and FAO, RDA/AI and UL. Competent organs have not evaluated concentrations of elements as Al, Cd, Ni, Co and Si in order to establish a tolerable upper intake level or RDA/AI for human. The lack of studies of adverse effects following excess intake of a nutrient does not mean that adverse effects do not occur.

Keywords
Leaf Chicory; Radicchio; Inductively Coupled Plasma Mass Spectrometry (ICP-OES).
intestinal worms and parasites to significant effect. Radicchio promotes bile production, which improves digestion, reduces cholesterol and can help lower blood pressure. Moreover, studies showed that its leaves are an excellent source of phenolic flavonoid antioxidants [9]. A recently published report reveals that radicchio hold moderate amounts of essential B-complex groups of vitamins, vitamin C, pyridoxine (vitamin B6) and thiamin (vitamin B1), niacin (B3) and vitamin K1 [10]. Radicchio also has folate (vitamin B9) which are known to provide neurological benefits [11]. Studies suggest that total intake of folate at or above the recommended dietary allowance (RDA) is associated with a reduced risk of Alzheimer’s disease [12].

Radicchio is used for the treatments of various disease. Its contents of calcium, copper, iron, magnesium, manganese, phosphorus, selenium and zinc are available in the database released by the United States Department of Agriculture (USDA) [13]. However, depending upon their geographical sources sometimes the contents of macroelements and microelements or heavy metals may differ in concentration and quantity of elements, which may lead to severe toxicity. Vegetables and fruits are subject to surrounding climatological conditions as well as mineral composition of soil as leaching, extremes of soil pH, soil limited, land leveling, high oil phosphate, soil compaction, tie-up in high organic soils by manure amendments and metal pollution in the environment [14].

Almost all types of soil known to soil scientists exist in Brazil. Brazil has a different soil from other countries [15]. Thus, the concentration and amount of elements detected in some plants may be different between countries and their regions. There is a gap in the knowledge about the composition of elements in the vegetables that needs to be filled. It’s necessary to know the levels of macro and microelements in vegetables to estimate their role as sources of these components in the human diet because, above the requirement elevated levels, these elements can pose a risk of adverse effects on health. For many laypeople was and still is hard to understand and accept such evidence. Therefore, it is important to have a look on good quality control of vegetables in order to protect consumers from contamination.

The macroelements are required in amounts greater than 100 mg/day and the microelements are required in amounts less than 100 mg/day [16]. Macroelements (sodium, potassium, calcium, magnesium, phosphorus) have certain key function in the living organisms. For human the microelements as zinc, iron, copper, chromium, molybdenum, selenium and cobalt are considered as essential and become harmful only at high concentration. In addition, the deficiency of certain elements also cause disease in human and plants. Thus, know the concentration of minerals in vegetables is very important and need to be screened for their quality control.

The aim of present work was to measure for the first time the macroelements and microelements in the leaf chicory used for human consumption by populations of the city Campo Grande. State of Mato Grosso do Sul, Midwest of Brazil. There are no studies published on chemical composition of leaf chicory in Brazil or others country. The macroelements and microelements content, after microwave digestion was determined by inductively coupled plasma - optical emission spectroscopy (ICP-OES). Results obtained of the concentration of macro and microelements were compared with the recommended dietary allowance (RDA), adequate intake (AI) and tolerable upper intake level (UL). The study focuses on the one hand on essential elements and on the other hand on non-essential elements which are considered as toxic for humans, covering in total K, Mg, Ca, P, Cr, Cu, Fe, Mn, Mo, Zn, Al, Cd, Ni, Co and Si.
Materials and Methods

Sample Collection
The leaf chicory (*Cichorium intybus* L.) were purchased from the wholesaler CEASA (Central Supply of Mato Grosso do Sul) in an urban area in the city of Campo Grande, Mato Grosso do Sul, with the coordinates 20°28'43"S and 54°38'28"W, altitude of 551 m. CEASA is a mixed economy company, administratively linked to Agency for Agrarian Development and Rural Extension of Mato Grosso do Sul. The purpose is to guide, discipline, distribute and commercialize horticultural products in the State of Mato Grosso do Sul. The Figure 1 has the Geographic coordinates of Campo Grande, State of Mato Grosso do Sul, Brazil.

![Figure 1: Map of Brazil showing the localization of the Midwest region of Brazil. Geographic coordinates of Campo Grande, State of Mato Grosso do Sul.](image1)

Radicchio prefers cool weather, fertile soil and humid. 'Radicchio' matures in approximately three months after seedling. Its wine-red color leaves with prominent white veins (Figure 2). According Ref. [6], different cultivars of radicchio are grown generally by the name of Veneto provincial cities.

Processing the Samples by Acid Digestion
The leaves of radicchio were oven dried at the temperature of 45 °C for 24 hours. The dried samples were then ground with a manual grinder into powder and sieve to get very fine powder. It was then weighed. The samples were digested using hot acid digester equipment (microwave digestion system Speedwave Berghof, Germany) applying 0.5 g from each sample with addition of 5 ml of nitric acid 65% (HNO₃) and 3 mL hydrogen peroxide 35% (H₂O₂), both of analytical grade at a temperature from 50 to 190 °C, heated until complete digestion. After digestion, samples were diluted to 100 mL with ultrapure water. Since the final acid concentration of the samples was quite high (4% HNO₃). All analyses were performed in triplicates.

Elemental analysis by ICP-OES technique
The concentrations of the elements in leaf chicory were determined by technique of inductively coupled plasma - optical emission spectrometer (ICP-OES) with a dual plasma (Thermo Scientific - iCAP 6000 Series). The system parameters were applied using the optimize source function, which automatically optimizes pump speed, nebulizer gas flow and RF power for the best signal. ICP-OES elemental analysis technique that uses the emission spectra of a sample to identify, and quantify the elements present. The emission line selected for the determination of elements (wavelength in nm) is shown in Table 1. The concentrations of the different elements in these samples were determined using the corresponding standard calibration curves obtained by using standard solutions of the elements of in-
Table 1. Method validation, ICAP 6000 series-duo, thermo scientific, pneumatic nebulization. Macroelements (nm) and linearity ($R^2$).

| Elements | Wavelength (nm) | Linearity ($R^2$) |
|----------|----------------|------------------|
| Macroelements: K  | 766.490   | 0.9136           |
|            Mg     | 285.213   | 0.2960           |
|            Ca    | 396.847   | 0.9900           |
|            Na    | 588.995   | 0.7911           |
|            P     | 178.284   | 0.9896           |
| Microelements: Cr | 267.716   | 0.9993           |
|            Cu    | 327.396   | 0.9962           |
|            Fe    | 239.562   | 0.9063           |
|            Mn    | 259.373   | 0.9999           |
|            Mo    | 202.030   | 0.9970           |
|            Zn    | 206.200   | 0.9957           |
|            Al    | 308.215   | 0.9763           |
|            Cd    | 228.802   | 0.9999           |
|            Ni    | 331.604   | 0.9987           |
|            Co    | 228.616   | 0.9999           |
|            Si    | 251.611   | 0.9121           |

Basic requirements of comparative

The dietary reference intakes (DRIs) are reference values developed and published by the Institute of Medicine (IOM) and may be used to assess whether diets provide enough nutrients or dietary components to meet requirements of healthy people without being excessive and reduce the risk of chronic disease [17]. They include both recommended dietary allowance (RDA), adequate intake (AI) and tolerable upper intake Level (UL). In Brazil, Brazilian Health Surveillance Agency (ANVISA) use the recommended value by DRI [18]. The values of the concentration of minerals obtained in the leaf chicory or radicchio were compared with the following recommended values for children (1-3 years) and adults (14-70 years):

- **RDA**: refer to the recommended daily levels of nutrients to meet the needs of 98% of healthy individuals in a particular age and gender group.
- **AI**: established when evidence is insufficient to develop an RDA and is set at a level assumed to ensure nutritional adequacy for a group (or groups) of apparently healthy people.
- **UL**: maximum daily intake unlikely to cause adverse health effects in 97.5% of healthy individuals in each life-stage and sex group.

When necessary, were considered the interval of maximum and minimum intake values established by the RDA/AI for men and women aged 14 to 70 years. In the absence of established RDA or data on adequate intake for children or adults, the results obtained within the framework of this study were compared with the estimates based on the regulatory limits of the WHO/FAO and published studies involving vegetables when available in the literature.

Nutrient content claims for “good source” and “high”

According to the standards established by Food and Drug Administration a claim about the level of a nutrient in a food in relation to the RDI or daily reference value (DRV) may only be made on the

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label or in labeling of the food if the claim is made in accordance with the general requirements for nutrient content claims. The standards established definitions are as follows [19]:

The terms “good source,” “contains,” or “provides” may be used on the label and in the labeling of foods, provided that the food contains 10-19% of the RDI or the DRV per reference amount customarily consumed.

The terms “high,” “rich in,” or “excellent source of” may be used on the label and in the labeling of foods, provided that the food contains 20% or more of the RDI or the DRV per reference amount customarily consumed.

Results and Discussion
Concentrations of the elements determined in the analyzed leaf chicory (Cichorium intybus L.) or radicchio are shown in Table 2. For some elements, there are one interval of maximum and minimum of the values established by the RDA/AI for men and women aged 14 to 70 years. In addition, values tolerable upper intake level (UL) and RDA/AI for children (1-3 y) also are listed in Table 2. In the present work, the concentration of macroelements in leaf Chicory decreases in the order: K > P > Ca > Mg > Na. As well as the concentration of microelements decreases in the order: Fe > Al > Si > Mn > Zn > Cu > Cr > Ni > Cd > Mo > Co. Among the various elements K, P, Mg, Al, Fe are found present at the major level. On the other hand, Cr, Mo, Ni and Co are at minor level. The results and discussions on the concentration of each element obtained in the leaf chicory in this manuscript are presented in the order in which they appear on Table 2.

Table 2. Elemental concentration (mg/100 g) of leaves of Cichorium intybus Linnaeus (leaf chicory) or Radicchio compared to recommended dietary allowances/adequate intake (RDA/AI) and values tolerable upper intake level (UL), both for children (1–3 y) and for adults (14–70 y) [17].

| Element | Our results Radicchio | RDA/AI* Children (1–3 y) [17] | UL Children (1–3 y) [17] | RDA/AI* Adults (14–70 y) [17] | UL Adults (14–70 y) [17] |
|---------|-----------------------|-----------------------------|--------------------------|-------------------------------|-------------------------|
|         | mg/100g   | mg/day  | mg/day  | mg/day  | mg/day  |
| Na      | 51.080    | 1,000*  | 1,500  | 1,300-1,500* | 2,200-2,300 |
| K       | 4,550.96  | 3,000*  | ND     | 4,700* | ND       |
| Ca      | 248.160   | 500*    | 2,500  | 1,000*-1,300* | 2,500 |
| Mg      | 124.02    | 80      | 65     | 355    | 355¹    |
| P       | 842.820   | 460     | 3,000  | 700-1,250 | 4,000 |
| Cr      | 0.0736    | 0.011*  | ND     | 0.020--0.030* | ND          |
| Cu      | 1.0456    | 0.34    | 1.0    | 0.89–0.90 | 8-10      |
| Fe      | 19.188    | 7       | 40     | 8–18   | 45       |
| Mn      | 6.2886    | 1.2*    | 2      | 1.6*–2.3* | 11        |
| Mo      | 0.0152    | 0.017   | 0.3    | 0.043–0.045 | 1.7-2.0  |
| Zn      | 2.281     | 3.0     | 7      | 8.0–11.0 | 34-40     |
| Al      | 14.602    | ND      | ND     | ND     | ND       |
| Cd      | 0.019     | ND      | ND     | ND     | ND       |
| Ni      | 0.0574    | ND      | 0.2    | ND     | 1.0      |
| Co      | 0.0149    | ND      | ND     | ND     | ND       |
| Si      | 6.78      | ND      | ND     | ND     | ND       |

¹: The UL for magnesium is determined by supplementation only and does not regard the ingestion from food or water; ND: Not determinable.
Microelements

Sodium (Na)

In Table 2, the concentration of Na detected in leaf chicory were 51.08 mg/100 g, and is the lowest concentration of all microelements obtained. Content of sodium accounts for only about 5.1 percent of adequate intake for children (1,000 mg/day of AI), while 3.4 to 3.9 percent of total intake for adults (1,300-1,500 mg/day of AI). After comparison, value of concentration of Na in the studied leaf chicory with those proposed by AI, it is concluded that the leaf chicory is not considered a source of sodium for children and adults.

Tolerable upper intake level (UL) for consuming of sodium in children and adults is 1,500 mg/day and 2,300-2,500 mg/100 g. Therefore, the results in Table 2 are below the values tolerable upper intake level (UL). World Health Organization (WHO, 2012) recommended a reduction to < 2 g/day sodium in adults. However, this recommended maximum level of intake of Na in adults should be adjusted downward based on the energy requirements of children relative to those of adults [20]. The Na concentration found in our work is below the stipulated values by the WHO and DRIs. High dietary sodium intakes increase the development of hypertension [21].

Potassium (K)

It is observed that amongst all the elements studied in the analyzed sample, potassium accumulation is the highest in leaf chicory than the concentration of other metals. The present study revealed that the concentration of K in leaf chicory was 4,550.90 mg/100 g, which correspond to 151.6% of AI for children (3,000 mg/day) and 96.82% of AI for adults (4,700 mg/day). After comparison, value of concentration of K in the studied leaf chicory with those proposed by AI, our results demonstrated that the leaf chicory is excellent source of potassium for children and adults.

The tolerable upper intake levels (UL) are not established for potassium in children and adults. Therefore, the available data are insufficient to establish a safe upper intake level for potassium. In European countries, daily intakes of K from the habitual diet generally do not exceed 5-6 g/day and has not been associated with any negative effects in healthy populations [22]. The WHO suggests for adults (≥ 16 years of age) a potassium intake of at least 3,510 mg/day, in which should be adjusted for children between 2 and 15 years of age, based on the energy requirements of children relative to those of adults [23]. In this way, leaf chicory concentration of potassium are above the values recommended by WHO. Have been that the higher potassium intake was associated with a lower risk of stroke [24].

Calcium (Ca)

Concentration of calcium detected in the leaf chicory were 248.160 mg/100 g (Table 2), which correspond to 49.6% of AI for children (500 mg/day) and 24.8% of AI for adults (1,000 mg/day). After comparison, value of concentration of Ca in the studied leaf chicory with those proposed by AI, our results demonstrated that the leaf chicory is excellent source of calcium for children and adults. Leaves of chicory is considering good source of potassium (19%), when comparing the AI for adults of 1,300 mg/day.

For all age groups, the results in Table 2 for Ca concentration are below the tolerable upper intake level (2,500 mg/day) for children and adults. Therefore, there is no risk of adverse effects in populations with low dietary calcium intake. In some studies, calcium have linked calcium intake to the prevention of colon cancer [25]. In Spanish children, results of studies suggested that there is a public health problem that must be addressed through nutrition education programs to increase intake of calcium-rich food and to correct the associated dietary pattern [26].

Magnesium (Mg)

In this study, in Table 2, a higher concentration of Mg was founded in the leaf chicory were 124.02
mg/100 g, which correspond to 155.01% of RDA for children (80 mg/day) and 34.9% of RDA for adults (355 mg/day). After comparison, value of concentration of magnesium in the studied leaf chicory with those proposed by RDA, our results demonstrated that the leaf chicory is excellent source of magnesium for children and adults.

According to results in Table 2, magnesium concentrations are above the tolerable upper intake level for children (65 mg/day) and below for adults (351 mg/day). Studies has been reported that a large portion of Americans do not consume the RDA for magnesium [27]. In fact, from 2001 to 2004, only 15% of Americans ages 2 years and older consumed the recommended servings of dairy products per day [28]. As in adults, it is considered that children are susceptible to the osmotic effects of nonfood sources of magnesium. From the adjusting the values for adults on a body-weight basis, established a UL for children at a magnesium intake of 5 mg/kg/day. Nonfood sources such as some magnesium salts used for pharmacologic purposes when consumed in excess cause adverse effects [29].

**Phosphorus (P)**

In Table 2, concentration detected of P in leaf chicory were 842.820 mg/100 g, which correspond to 183.2% of RDA for children (460 mg/day) and 67.42-120.4% of RDA for adults (700-1,250 mg/day). After comparison, value of concentration of phosphorus in the studied leaf chicory with those proposed by RDA, the present study indicates that the leaf chicory is excellent source of P for children and adults.

The UL for phosphorus is 3,000 mg/day for children and 4,000 mg/day for adults. In this case specifically, the contents of phosphorus obtained of the leaf chicory were well below the permissible limit. Therefore, according the values of ULs, the contents of phosphorus obtained of the leaf chicory is not likely to cause adverse health effects. Phosphorus is found in smaller amounts in vegetables and fruit [30]. However, our results show that leaf chicory has a phosphorus concentration greater than other vegetables. Therefore, it is interesting to encourage its consumption by the population. According controlled trials with young women found no adverse effects of a diet rich in phosphorus of up to 3,000 mg/d on bone-related hormones and biochemical markers of bone reabsorption [31].

**Macrolelements**

**Chromium (Cr)**

Concentration of Cr detected in the leaf chicory were 0.0736 mg/100 g (Table 2), which correspond to 669.09% of AI for children (0.011 mg/day) and 368.0-245.0% of AI for adults (0.020-0.030 mg/day). After comparison, value of concentration of chromium in the studied leaf chicory with those proposed by AI, our results demonstrated that the leaf chicory is excellent source of Cr for children and adults.

Tolerable upper intake level (UL) for consuming of chromium in children and adult is not established yet. The permissible limit set by FAO/WHO (1984) in edible plants was 0.002 mg/100 g [32]. After comparing the value of concentration of chromium in the studied leaf chicory with those proposed by FAO/WHO (1984), it is concluded that the concentration of chromium in leaf chicory were recorded above the permissible limit set by FAO/WHO. Chromium at an elevated concentration it is toxic for both plant and animals. However, the beneficial effects of supplemental Cr in individuals with type 2 diabetes were observed at levels higher than the upper limit of the estimated safe and adequate daily dietary intake [33].

**Copper (Cu)**

In Table 2, the concentration of Cu detected in leaf chicory were 1.0456 mg/100 g. Content of copper accounts for only about 307.3 percent of RDA for children (0.34 mg/day of RDA), while 117.4 to 116.0 percent of RDA for adults (0.89-0.90 mg/day of RDA). After comparison, value of concentration of copper in the studied Leaf chicory with those
proposed by RDA, our results demonstrated that the leaf chicory is an excellent source of Cu for children and adults.

The tolerable upper intake level (UL) for consuming of Cu in children and adults is 1.0 mg/day and 8.0-10.0 mg/100 g. Therefore, the results in Table 2 are below the values tolerable upper intake level (UL), there is no risk of adverse effects for adults and children. However, the permissible limit of copper set by FAO/WHO (1984) in edible plants is 0.3 mg/100 g. Comparing the concentration of copper obtained in the leaf chicory with the values established by the FAO/WHO (1984), it is observed that the leaf chicory accumulate Cu above this limit. Copper is an essential trace element that is vital to the health in humans. [34]. According recent studies, there is an association between a diet rich in copper and iron and Alzheimer's disease [35].

Iron (Fe)
Concentration of Fe detected in the leaf chicory were 19.188 mg/100 g (Table 2), which correspond to 274.0% of RDA for children (7.0 mg/day) and 239.7-106.5% of RDA for adults (8.0-18.0 mg/day). After comparison, value of concentration of iron in the studied leaf chicory with those proposed by RDA, our results demonstrated that the leaf chicory is an excellent source of Fe for children and adults.

The tolerable upper intake level (UL) for iron is 40 mg/day for children and 45 mg/day for adults. Comparing our results with those values of UL, it observed the contents of iron obtained of the leaf chicory were well below the permissible limit by UL for children and adults. Therefore, according the values of ULs, the contents of iron obtained of the leaf chicory is not likely to cause adverse health effects. On the other hand, the permissible limited set by FAO/WHO (1984) in edible plants was 0.2 mg/100 g and it was found that leaf chicory accumulated iron above this limit. Although the vegetarians had diets nearer to the recommended diet with a lower fat and salt content, higher total daily iron intake than those who eat meat, fish or poultry. For men, recommended intakes of iron may need to be higher for vegetarians [36].

Manganese (Mn)
Concentration of Mn detected in the leaf chicory were 6.288 mg/100 g (Table 2), which correspond to 524.0% of AI for children (1.2 mg/day) and 393.0-273.03% of AI for adults (1.6-2.3 mg/day). After comparison, value of concentration of Mn in the studied leaf chicory with those proposed by AI, our results demonstrated that the leaf chicory is an excellent source of manganese for children and adults.

Manganese is a mineral element that is both nutritionally essential and potentially toxic for human health in high levels [37]. Studies in children have suggested that extremely high levels of manganese exposure may changes in behavior and decreases in the ability to learn and remember [29]. In fact, the effect of manganese in drinking water on children's IQ (intelligence quotient) have been studied and proven [38].

Molybdenum (Mo)
Concentration of molybdenum detected in the leaf chicory were 0.0152 mg/100 g (Table 2), which correspond to 89.4% of RDA for children (0.017 mg/day) and 35.3-33.70% of RDA for adults (0.043-0.045 mg/day). After comparison, value of concentration of molybdenum in the studied leaf chicory with those proposed by RDA, our results demonstrated that the leaf chicory is an excellent source of molybdenum for children and adults.
The tolerable upper intake level (UL) for consuming of molybdenum in children and adults is 0.30 mg/day and 1.7-2.0 mg/day. After comparison, value of concentration of molybdenum in the studied leaf chicory with those proposed by UL, it observed that the results in Table 2 are below the values tolerable upper intake level (UL), there is no risk of adverse effects for adults and children.

Little is known about the effects of elevated molybdenum levels on living systems and the value of tolerable upper intake level depend on each country. According to US food and nutrition board (FNB, 1989) and references therein [39], the UL is six times the mean estimated intake of 100 µg Mo/day for adults in 11 different countries [40], and exceeds the upper value of intakes for the Netherlands (96 µg/day), Sweden (260 µg/day), the United Kingdom (400 µg/day), Germany (500 µg/day), and Finland (150 µg/day) [41-42].

Zinc (Zn)
In Table 2, concentration of zinc detected in the leaf chicory were 2.281 mg/100 g, which correspond to 76.0% of RDA for children (3.0 mg/day) and 28.51-20.73% of RDA for adults (8.0-11.0 mg/day). After comparison, value of concentration of zinc in the studied leaf chicory with those proposed by RDA, our results demonstrated that the leaf chicory is excellent source of zinc for children and adults.

The tolerable upper intake level (UL) for consuming of zinc in children and adults is 7 mg/day and 34-40 mg/day. After comparison, value of concentration of zinc in the studied leaf chicory with those proposed by UL, it observed that the results in Table 2 are below the values tolerable upper intake level (UL), there is no risk of adverse effects for adults and children.

Zinc is an important factor in the metabolism of neurotransmitters, prostaglandins, and for maintaining brain structure and function. In fact, recent studies showed the role of zinc in the treatment of hyperactivity disorder in children [43]. However, the recommendation of zinc is beneficial in the treatment of several disorders, such as several pro-inflammatory conditions and cancer [44].

Aluminum (Al)
Aluminum contents were 14.60 mg/100 g for the leaf chicory respectively. Until now, there are not recommendation (RDA) and adequate intake for aluminum (Al). As well as there are not values tolerable upper intake level (UL). On the other hand, mean exposures of the adult population to Al from overall diet including additives varied ranged from 1.6 mg/day in French studies [45], United Kingdom (1.3 mg/kg bw/week for toddlers (1.5-4.5 years)) [46], and Mainland China (4.0 mg/kg bw/week for 60-kg adults) [47]. It was found that the mean dietary exposure of the whole Chinese population to intake of aluminum from Al-containing food additives were 2.103-2.903 mg kg-1 bw week-1 for children, exceeding the provisional tolerable weekly intake (PTWI) [48].

After comparison, value of concentration of Al in the studied leaf chicory with those proposed by above studies, it observed that the results in Table 2 are above these values. The tolerable upper intake levels are scarce in the literature and differentiate between countries. In this way we can not assure for sure if the aluminum in the concentration obtained in the leaf chicory can cause or not toxicity. Compared to the PTWI of 7 μg/(kgbw·week) proposed by the WHO the dietary intake of cadmium was rather high.

Cadmium (Cd)
According to data in Table 2, the contents of Cd was 0.019 mg/100 g in the leaf chicory. A safe recommended dietary allowance or adequate intake for Cd hasn’t been set yet. There is not tolerable upper intake level for consuming of Cd in adults and children. However, the FAO/WHO (1984) has set in edible plants was 0.021 mg/100 g [32]. After comparison, cadmium limits in the leaf chicory
with those by FAO/WHO (1984) it was found that leaf chicory have Cd below this limit.

There is little general agreement about acceptable safety limits for Cd intake. However, the dietary intake of Cd was studied among children and adults from Germany. In those studies, the daily Cd intakes for small children (mean age: 1.8 years) were 0.17 μg/(kgbw·day) [range: 0.02-1.62 μg/(kgbw·day)] and for adults (mean age: 40.9 years) were 0.37 μg/(kgbw·day) [range: 0.05-1.32 μg/(kgbw·day)]. Compared to the PTWI of 7 μg/(kgbw·week) proposed by the WHO the dietary intake of Cd was rather high. The authors conclude that the Cd exposure of the population needs to be reduced in order to minimize the risk of adverse health effects related to this metal [49].

**Nickel (Ni)**

Nickel concentration detected in the leaf chicory were 0.0574 mg/100 g in Table 2. Agriculture organization (FAO) of the United Nations in 1984 the permissible limit inedible plants is 0.163 mg/100 g [32]. From of information about metal limit in edible plants proposed by FAO/WHO (1984) it is found that the leaf chicory accumulates Ni below this limit.

The UL for consuming of Ni in children and adults was 0.2 mg/day and 1.0 mg/day. After comparison, value of concentration of nickel in the studied leaf chicory with those proposed by UL for children and adults, it observed that the results in Table 2 are below the values tolerable upper intake level. Nickel compounds are known as human carcinogens, but the fundamental mechanisms are still not fully understood [50]. The Nickel present in some food is considered the major source of exposure for children and adults. In people who are sensitive to nickel, dermatitis may occur [51].

**Silicon (Si)**

In Table 2, concentration of Si detected in the leaf chicory were 6.78 mg/100 g. A safe recommended dietary allowance or adequate intake for silicon hasn’t been set yet for human. As well as there is not UL for consuming of Si in adults and children.

The daily intake from the British diet has been estimated to 20-50 mg corresponds to 0.3-0.8 mg/kg body weight/day in a 60 kg person [53]. However, the result in Table 2 for silicon is within limit the daily intake from the British diet.

Silicon has been used as an additive in the food and beverage industry. There are discusses the possible biological potential of the metalloid silicon as bioavailable orthosilicic acid and the potential beneficial effects on human health [54]. Although the main source of silicon for humans is the diet, the bioavailability of silicon from solid foods is not fully understood. It is present in the pancreas, where it plays an important role in the production of insulin.
Conclusions

For the first time, determination of macroelements and microelements were measured in the leaf chicory. It is very important to know the level of microelements and macroelements in leaf chicory to estimate their role as source of these components in the human diet. The data obtained in the manuscript on elemental composition of leaf chicory demonstrated that this vegetable is excellent source of K, Ca, Mg, P, Cr, Cu, Fe, Mn, Mo and Zn for children and adults. On the other hand, leaf chicory are not considered a source of sodium for children and adults.

The concentration of some elements as Na, Ca, P, Cu, Fe, Mo, Zn, Cd and Ni obtained in the leaf chicory are below of recommended limit by WHO and/or values tolerable upper intake level. However, the concentration of K, Cr, Fe, and Al are above the limit recommended limit by FAO/WHO (1984) and others countries.

Silicon is within limit the daily intake from the British diet. After comparison, concentration of cobalt in the leaf chicory with those by agency for toxic substances and disease registry (ATSDR) it was found that leaf chicory have Co above this value.

A safe recommended dietary allowance or adequate intake for Al, Cd, Ni, Co and Si hasn’t been set yet for human. As well as there is not tolerable upper intake level (UL) for consuming of those elements in adults and children. There are no established a permissible criteria for level of silicon in vegetables. Silicon is within limit the daily intake from the British diet. Leaf chicory has macro and microelements in ample amount; it can be used as a good natural dietary supplementary. However, the use of vegetables acquired in public markets or street market, by urban populations cannot be ignored because the element toxicity risk assessment is bases rather on habitual than on incidental intake. In fact, some essential nutrients can be toxic at some level above the requirement and allowable limits set by WHO and FAO, RDA and UL.

For some nutrients, such as aluminum, cadmium, nickel, cobalt, chromium, and silicon, there may be inadequate data on which to develop a UL. The lack of studies of adverse effects following excess intake of a nutrient does not mean that adverse effects do not occur.

Conflict of interest disclosure

The authors declare that there is no conflict of interest regarding the publication of this paper.

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