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Effect of Processing Methods on Heavy Metal Concentrations in Commonly Consumed Green Leafy Vegetables Available in Sri Lankan Market

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Abstract: Green leafy vegetables are considered as a good source of nutrients (vitamins, minerals, fibers and phytochemicals), particularly among urban populations dependant on plant based diets. Recently, several research studies conducted in and around Colombo District, Sri Lanka, have reported increased trace element concentrations in green leafy vegetables collected from production and market sites. Therefore, the present study was conducted with the aim of studying the effect of three food processing techniques in reducing the toxic elements present in green leafy vegetables commonly consumed in Sri Lanka. Five types of green leafy vegetables ["Mukunuwenna" (*Alternanthera sessilis*), "Thampala" (*Amaranthus viridis*), "Nivithi" (*Basella alba*), "Kohila Leaves" (*Lasia spinosa*) and "Kankun" (*Ipomoea aquatica*)] were randomly collected from ten different markets sites located in and around Colombo District, Sri Lanka. Each type of green leafy vegetable sample was subjected to three processing treatments (raw, cooked and stir-fried) and analyzed for five trace elements (Ni, Cd, Cr, Pb and Cu), using the Atomic Absorption Spectrophotometry. The average concentrations (mg kg^{-1}, dry weight basis) of metals detected in raw, cooked and stir-fried green leafy vegetable samples were as follows: Ni (2.93±2.88, 2.28±1.44, 2.05±1.31), Cd (0.30±0.25, 0.20±0.19, 0.20±0.18), Cr (2.45±1.78, 2.34±1.62, 2.24±1.64), Pb (0.59±0.42, 0.48±0.39, 0.44±0.38) and Cu (11.30±4.21, 9.73±3.69, 9.23±4.48). The results showed no significant differences in heavy metal contents among three processing methods ($p<0.05$). Therefore, the type of processing method has a minimal effect in reducing the heavy metal contents of green leafy vegetables.

Key words: Heavy metals, green leafy vegetables, raw, cooked, stir-fried

INTRODUCTION

Metals are natural components of the earth's crust. However the distribution of heavy metals in the environment can vary between different regions based on the specific characteristics of the metal and by the influence of environmental factors (Khlifi and Hamza-Chaffai, 2010). Heavy metals come into the environment as a result of natural and anthropogenic causes. Eg: natural weathering of rocks, mining, deterioration of soils, industrial and vehicle emissions, urban effluents and surface runoff, fertilizers and pesticides, atmospheric contamination etc., (Ming-Ho, 2005). For most individuals the major route of exposure to these toxic heavy metals is through the diet (food and water). The contamination sequence of heavy metals usually follows a cyclic pathway: industry, air, soil, water, foods and human. It is widely-known that the long-term exposure to toxic heavy metals at relatively low doses can cause adverse health effects in mankind (Agency for Toxic Substance and Disease Registry (ATSDR), 2004, 2007, 2008; Castro-Gonzalez and Mendez-Armenta, 2008). Therefore, there has been constant interest, about exposures, intakes and absorption of heavy metals by humans.

In recent years, numerous research projects have been conducted in various countries, to explore the impact of various cooking and processing methods on toxic metals in food. Different foodstuffs have been considered for those studies including rice, fruits and vegetables, fish and other sea foods. A considerable number of studies have recorded significant reductions of the heavy metals in foods after cooking; however other studies reported increased metal concentration after processing.

Each trace metal has an appreciable role in either aquatic or terrestrial food chains. For example, mercury has a less significant role in terrestrial food chains, while it is predominantly detected in sea foods and other marine organisms (Clarkson, 1994; Jures and Blanus, 2003). Inorganic arsenic considered as the most toxic

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chemical species of arsenic found in food and drinking water and terrestrial foods typically reported low levels of arsenic (Guha Mazumder *et al*., 1998; Smith *et al*., 1998; Tondel *et al*., 1999; D'iaz *et al*., 2004; European Commission, 2004). In contrast to mercury and arsenic, cadmium, lead and other toxic elements enters the human diet primarily through terrestrial pathways. Edible plant crops served as the predominant food source of cadmium for humans and according to literature majority of human Cd uptake (about 70%) comes through the vegetables (Ryan *et al*., 1982; Chen *et al*., 2012). As mentioned by Chary *et al*. (2008), cadmium has increased mobility and phytoavailability and comparatively poor adsorption in soils. Due to its high mobility in soil, bioaccumulation of cadmium in plant-based foodstuffs is usually high, compared with the other trace metals (Lebeau *et al*., 2002; Satarug *et al*., 2003; Cui *et al*., 2005; Azizur and Hiroshi, 2011). In comparison to cadmium, lead has less transfer coefficients and it can strongly bind to the soil colloids. According to previous studies, the accumulation of lead in plants usually happens when the soil lead concentration is high (Chary *et al*., 2008). Compared with other aerial parts, majority of lead is retained in the roots of the plants. Nevertheless, the plant-based foods are considered as the major source of human dietary lead intake.

As a generation that shows more concern towards health, people nowadays are paying more attention to consuming a wholesome diet. It has been very common among Sri Lankan households to incorporate raw and cooked forms of green leafy vegetables into their daily diets, for nutritional purposes. Green leafy vegetables (GLV) are known to be rich in dietary fiber, vitamins, minerals and disease-fighting phytochemicals. According to the research studies carried out recently, it was reported that the most popular types of GLV cultivated and consumed in and around Colombo District, Sri Lanka, were contaminated with toxic heavy metals (Kananke *et al*., 2014, 2015; Premarathna *et al*., 2011). Therefore, the study was designed with the objective of assessing the effect of three processing conditions on five toxic elements present in five popular types of GLV consumed in and around Colombo District, Sri Lanka.

MATERIALS AND METHODS

Sample collection: Five key types of GLV cultivated and consumed in Sri Lanka, including "Mukunuwenna" (*Alternanthera sessilis*), "Nivithi" (*Basella alba*), "Thampala" (*Amaranthus viridis*), "Kohila Leaves" (*Lasia spinosa*) and "Kankun" (*Ipomoea aquatica*) were randomly sampled from ten different market sites (Kolonnawa, Wellampitiya, Piliyandala, Kesbewa, Kottawa, Bandaragama, Kahathuduwa, Delgoda, Pettah, Delkanda) located in and around Colombo District, Sri Lanka. The details of the test green leafy vegetables are given in Table 1.

A total of 50 samples (each five types of GLV from ten different market sites) were collected over a period of three months during January-March, 2015. All the collected samples were separately packed up in polyethylene bags and brought to the laboratory for sample treatment and analysis.

Sample preparation: The GLV samples collected (Fig. 1) were sorted and washed with clean tap water to remove the air-borne pollutants, soil and other adhered particles on the surface of the vegetables. The cleaned edible portions of each single sample were divided into three portions (200 g each). One portion was analyzed as fresh (raw) sample and the other two portions were prepared by cooking and stir-frying as described below: Preparation of GLV by cooking: 200 g of washed and sorted GLV sample was finely cut and mixed with 100 mL of coconut milk and cooked by moderate heating in an open stainless steel pan for 10-15 min.

Preparation of GLV by stir-frying: 200 g of washed and sorted GLV sample was finely cut and fried with 25 mL of boiling coconut oil in an open stainless steel frying pan for 10-15 min, with occasional stirring.

Estimation of heavy metals: Heavy metal concentrations of each test portions were determined according to the AOAC 999.11 procedure as described below (AOAC, 2002).

Pre-treatment: Test portions were dried in a drying oven, at 105°C, until obtained a constant weight then cooled to room temperature, crushed by means of a clean pestle and mortar to obtain homogenized samples. The ground samples were stored at ambient temperature in airtight sealed polyethylene bags until required for analysis.

Dry digestion: Dried GLV samples (about 1-3 g) were placed in cleaned porcelain crucibles and placed in a muffle furnace. The furnace temperature was slowly increased up to 450°C at a rate of not more than 50°C/h. The samples were ashed for more than 8 hrs until a white ash residue was obtained. The ash was digested in 5 mL of 6 M HCl and the acid was evaporated on water bath. Resulting residue was dissolved with 10-30 mL of 0.1 M Conc. HNO_3 acid and let stands for 1-2 h. Then the stirred solutions in the crucibles were filtered and transferred in to 50 mL volumetric flasks and made up to the mark with distilled water and shaken to mix well. The resulting sample solutions were taken for the estimation of heavy metal concentrations.

Table 1: Green leafy vegetables sampled from production sites

Local Name	English name	Scientific name	Edible part
Mukunuwenna	Sessile alligatorweed, Sessile joyweed	*Alternanthera sessilis*	Leaves and young stem
Nivithi	Malabar spinach	*Basella alba*	Leaves and young stem
Thampala	Chinese spinach, Green amaranth	*Amaranthus viridis*	Leaves and young stem
Kankun	Water morning glory	*Ipomoea aquatica*	Leaves and young stem
Kohila	Lasia	*Lasia spinosa*	Young leaves and stem

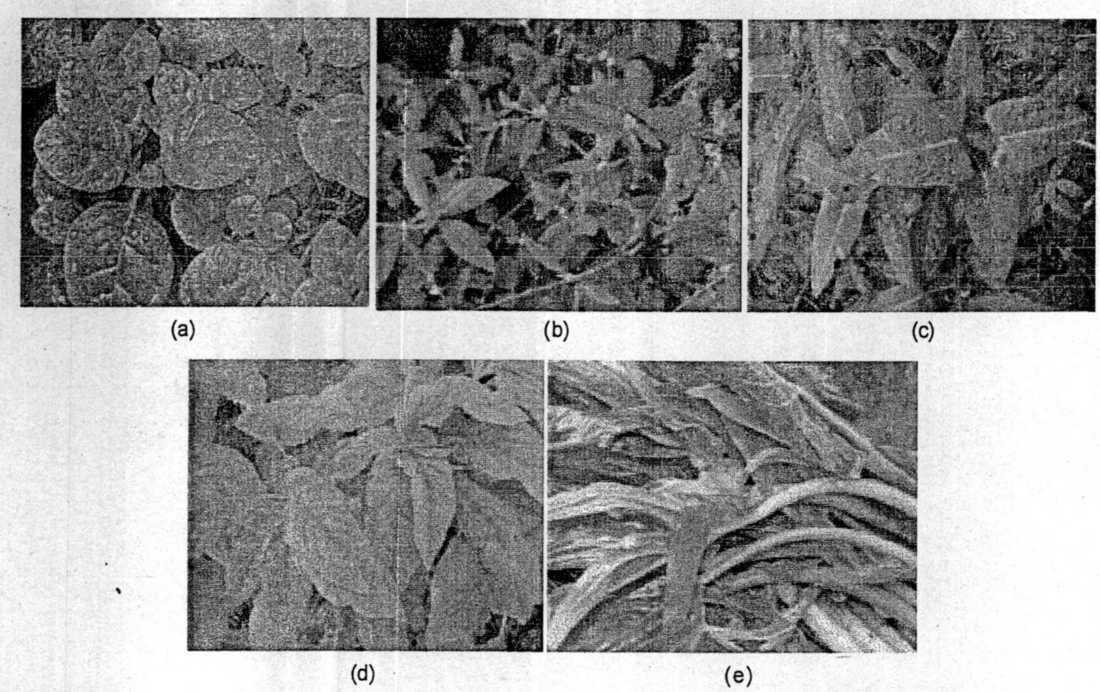

Fig. 1: Types of GLV collected from the market sites (a) Nivithi (b) Mukunuwenna (c) Kankun (d) Thampala (e) Kohila Leaves

Preparation of standards: All chemicals used in the experiment were purchased from the Sigma Aldrich Company, USA and all the reagents used were of analytical (trace-metal) grades. Working standard solutions of nickel (Ni), lead (Pb), copper (Cu), chromium (Cr) and cadmium (Cd) were prepared from the stock solutions having 1000 ppm of metal in 65% (w/w) nitric acid. The calibration curves were prepared for each individual element using linear correlation by least square method. Blank readings were also taken and appropriate corrections were made, while calculating the concentration of various elements.

Analysis of heavy metals: Trace element concentrations were determined by the Graphite Furnace (Ni, Cd, Cr and Pb) and Flame (Cu) Atomic Absorption Spectrophotometer (Thermo-Scientific ICE 3000 series). Wavelength, gas mixture/temperature program and other instrumental parameters were adjusted according to the guidelines provided in the instrument manual.

Calculation of heavy metals: Following formula was used to calculate the concentration of each metal in the test samples:

$$C = \frac{(a-b)}{w} \times v$$

where, C: Concentration of the metal in the test sample (mg/kg), a: Concentration of the metal in the test solution (mg/L), b: Mean concentration of the metal in the blank solution (mg/L), V: Volume of the test solution (mL), W: Weight of the test portion (g). Dilution factors were also considered in case of sample dilution.

Statistical analysis: Descriptive statistics and statistical significance of collected data were analyzed by ANOVA using Minitab 14.0 and Excel computer packages.

RESULTS AND DISCUSSION

The concentrations of heavy metals (Ni, Cd, Cr, Pb and Cu) detected in raw, cooked and stir-fried samples of the five types of GLV are presented in the Table 2. The mean values of Ni, Cd, Cr, Pb and Cu contents in raw, cooked

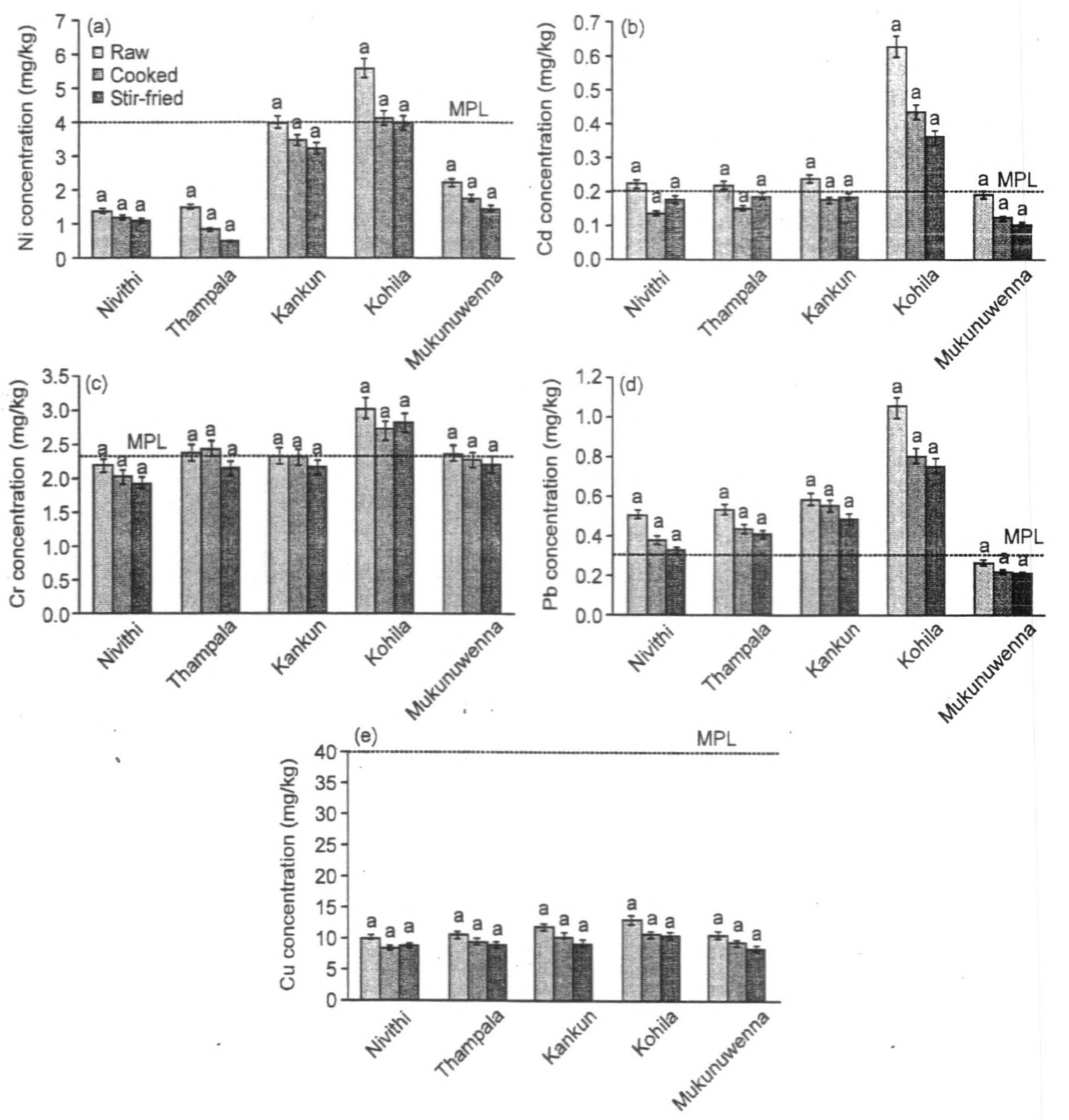

Fig. 2: Heavy metals (a) Nickel (b) Cadmium (c) Chromium (d) Lead and (e) Copper concentrations in raw, cooked and stir-fried forms of GLV. Letters with same characters are not significantly different at $p<0.05$ for each metal. MPL-Maximum Permissible Limit

and stir-fried GLV samples were analyzed by one-way analysis of Variance (ANOVA). The difference was considered statistically significant at the level of $p<0.05$. In almost all the five types of GLV, highest concentrations of metals were detected in the raw samples. When the GLV were subjected to cooking and stir-frying, generous reduction in the trace elements were noticed. However, the results were not significant at $p<0.05$ (Fig. 2). Therefore, the different processing techniques have minimal effects in reducing the heavy metal levels in GLV and this may be due to the difficulty of eliminating trace elements by either washing or heat processing.

In processed and un-processed Mukunuwenna samples, trace metals (except for Cr in raw Mukunuwenna samples) were detected below the WHO/FAO (2003) safe limit for human consumption. In Nivithi, raw samples reported Ni, Cr and Cu levels, well below the safe limits, in contrast to Cd and Pb. Cd contamination in Nivithi samples were reduced below the permissible level after being subjected to cooking and stir-frying techniques. However, the Pb contamination of Nivithi samples remained above the safe limit even after processing. In raw Thampala samples, the estimated average concentrations of Cd, Cr and Pb were higher than the WHO/FAO permissible

Table 2: Average concentrations of heavy metals detected in raw, cooked and stir-fried GLV

GLV	Treatment	Ni	Cd	Cr	Pb	Cu
Mukunuwenna	Raw	2.20±1.04	0.19±0.11	2.37±1.59	0.26±0.39	10.69±4.50
(n = 10)	Cooked	1.77±0.84	0.12±0.07	2.27±1.57	0.22±0.34	9.39±4.22
	Stir-fried	1.46±1.03	0.10±0.06	2.20±1.54	0.21±0.34	8.45±4.17
Nivithi	Raw	1.38±1.14	0.22±0.12	2.18±1.62	0.51±0.16	10.17±2.82
(n = 10)	Cooked	1.18±1.13	0.14±0.11	2.00±1.37	0.38±0.19	8.41±3.19
	Stir-fried	1.09±1.13	0.18±0.11	1.92±1.34	0.33±0.12	8.83±4.46
Thampala	Raw	1.49±1.30	0.22±0.14	2.37±2.10	0.53±0.35	10.63±3.06
(n = 10)	Cooked	0.83±0.64	0.15±0.10	2.43±1.93	0.44±0.36	9.57±3.13
	Stir-fried	0.49±0.55	0.19±0.13	2.14±1.84	0.41±0.33	8.99±3.45
Kankun	Raw	4.01±3.64	0.24±0.23	2.32±1.71	0.59±0.43	11.91±5.89
(n = 10)	Cooked	3.48±3.12	0.18±0.20	2.30±1.46	0.56±0.41	10.56±4.16
	Stir-fried	3.23±3.77	0.19±0.17	2.16±1.43	0.49±0.42	9.32±6.51
Kohila	Raw	5.59±4.61	0.63±0.29	3.02±2.07	1.05±0.36	13.12±4.20
(n = 10)	Cooked	4.12±4.17	0.44±0.23	2.70±1.95	0.81±0.41	10.71±3.89
	Stir-fried	3.98±3.96	0.36±0.30	2.80±2.12	0.76±0.43	10.57±3.85
All samples	Raw	2.93±2.88	0.30±0.25	2.45±1.78	0.59±0.42	11.30±4.21
(n = 50)	Cooked	2.28±1.44	0.20±0.19	2.34±1.62	0.48±0.39	9.73±3.69
	Stir-fried	2.05±1.31	0.20±0.18	2.24±1.64	0.44±0.38	9.23±4.48
WHO/FAO	Safe Limit	4*	0.2	2.3	0.3	40

*According to the Food and Nutrition Board, Institute of Medicine (2010)

Table 3: Previous research studies regarding the effect of processing methods on heave metal contents of foodstuffs

Food	Heavy metal	Processing method	Reduction	Reference
Green leafy vegetables	Ni, Cd, Cr, Pb, Cu	Cooking and Stir-frying	Not significant	Present study
Pasta	Ni, As, Cd, Pb	Milling and other pasta making processes	50-60%	Cubadda *et al.* (2003)
Rice	Cd, Pb, Cr, Ni, Co	Kateh and Pilaw	Not significant	Naseri *et al.* (2014)
Mushroom	Pb, Cd	Raw, Cooked, Fried, Micro-waved, Sliced	Significant	Ziarati *et al.* (2013)
Vegetables	Zn, Cd, Cr	Cooking time	Not significant	Joshua *et al.* (2012)
Vegetables and cereals	As	Cooking with distilled water	Significant	Diaz *et al.* (2004)
String bean and potato	As	Frying	Not significant	Perello *et al.* (2008)
Fish	Cr, Ni	Grilling and microwaving	Significant	Ersoy (2011)
Fish	Cr	Microwaving	Significant	Devesa *et al.* (2001)
Cray fish	Cd, Ni, Co	Cooking	Significant	Jorhem *et al.* (1994)
Fish	Pb, Cd	Canning	Significant	Ganjavi *et al.* (2010)

limits, in comparison to Ni and Cu. Further, the Cd levels in processed Thampala samples were reduced below the safe limit after being processed, in contrast to Pb. However, the mean concentration of Cr in cooked Thampala samples have increased above the level of Cr detected in raw samples. In Kankun, Ni, Cd, Cr and Pb reported elevated concentrations (above safe limits) before subjected to processing. The mean concentration of Pb, remained above the permissible guideline, even after subjecting to cooking and stir-frying, unlike Ni, Cd and Cr. Raw samples of Kohila have reported the highest contamination for all the five metals among the five GLV and the estimated levels of Ni, Cd, Cr and Pb levels were well above the WHO/FAO safe limits. Even after cooking or stir frying, the levels of Ni, Cd, Cr and Pb were not reduced below the permissible levels. In most samples stir-frying has reduced the levels of heavy metals than in cooking, except for Cd contents in stir-fried samples of Nivithi, Thampala and Kankun.

Cooking methods (boiling, steaming, frying etc.) can change the levels of toxic metals through various means, including the evaporation of water and volatile components, solubilization of the element and also by metal binding to the other macronutrients present in the food item such as carbohydrates, lipids and proteins. Since heavy metals cannot be evaporated or disintegrated in to non-toxic compounds, the metals which are detached from the foodstuffs during the processing treatment (frying, boiling, or canning) should be definitely migrate to the cooking medium (frying oil, boiling water or cooking stocks etc.).

Over the years, numerous studies have been performed in various parts of the world, to study the effects of different cooking and food processing techniques on the levels of toxic metals in different types of food products (fish and seafood, meat, fruits, vegetables, mushrooms, rice etc.). Some of the research findings have indicated significant reductions of the trace elements in foods after cooking or processing, while the other studies have reported elevated metal concentration after processing (Table 3).

According to previously conducted research, precooking methods (peeling of the outer layers of plant products, milling and grinding of cereal grains and some cooking methods) can remove some of the toxic elements originally present in the food. Cubadda *et al.* (2003), reported significant heavy metal reduction (nickel>arsenic>cadmium>lead) in pasta making process after subjecting to milling and other technological processes. Cooking removed considerable amounts of toxic metals present in pasta samples, with a mean reduction of 50 to 60%. As explained by the authors, most of the metals were present in the outer layers of the kernel and milling has reduced significant amount of metals present in the grains. Naseri *et al.* (2014) studied the effects of two cooking methods on the concentrations of some heavy metals (Cd, Cr, Pb, Ni and Co) in certain rice brands available in Iranian market. In this study, concentrations of Cd, Pb, Cr, Ni and Co, were measured in three brands of imported rice available in Shiraz-Iran market, after subjecting to two cooking methods (Kateh and Pilaw). According to results, heavy metals were detected in raw and cooked rice grains. Although, cooking of the rice grains reduced the content of heavy metals, the effect of cooking methods was not significantly different in their study. Ziarati *et al.* (2013) investigated the effect of cooking method on Lead and Cadmium contents in commonly consumed packaged mushroom (Agaricus bisporus) in Iran. The mushroom samples showed different heavy metal concentrations based on the experimental cooking method. Results revealed that the average lead and cadmium content in mushroom samples ranked from low to high concentration as follows for different cooking methods: Raw<Cooked< Fried<Microwaved<Sliced. Joshua *et al.* (2012) studied the effect of cooking time on mineral compositions of some local leafy vegetables such as Fluted Pumpkin (*Telifaira occidentalis*), Water Leaf (*Talinum fruticosum*), Bitter Leaf (*Vernonia amygdalina*) and Spinach (*Spinacia oleracea*). The results of the levels of Zn, Cd and Cr on the studied vegetables showed that increase in cooking time did not significantly affect the content of the minerals. The high amount of cadmium and chromium contained in spinach were mentioned to be due to the respective site of cultivation. D'iaz *et al.* (2004) examined the effect of different cooking methods frequently applied to cereals and vegetables (boiling, stewing, pureeing, baking etc.) on the arsenic concentration. According to the results, cooking foods with distilled water has significantly reduced the arsenic level of the raw food item. Authors justified that was because, the heat treatment accelerates breakdown of bonds between arsenic and food molecules and thereafter, assists its solubilization in washing and/or boiling water. Perello *et al.* (2008) investigated the impact of general Spanish cooking practices on toxic elements (mercury, lead, arsenic, cadmium) present in various foodstuffs. Results affirmed that boiling has significantly reduced the arsenic contamination in vegetables (string bean and potato), unlike frying. However, the type of cooking method showed no significant differences in other metals.

Many previous studies conducted in other countries have reported significant reductions in toxic elements present in high protein foods (fish, shrimps, mushrooms etc.) after subjecting to various processing methods, unlike plant based foods. Ersoy (2011) studied the effect of various cooking techniques on trace element levels in African catfish. The Ni concentration was decreased in grilled fish. In addition a significant reduction of Cr was also reported in grilled and microwave-cooked fish. In Devesa *et al.* (2001) found decreased Cr levels in African catfish fillets after grilling and microwaving, in comparison to raw fillets. A research conducted by Jorhem *et al.* (1994) also reported that the Cd, Ni and Co levels in crayfish reduced after cooking. The above studies have indicated that the application of heat during thermal processes hastens protein degradation, which may affect the levels of toxic elements in food. Ganjavi *et al.* (2010) studied the effects of canning on the levels of Cd and Pb in yellowfin tuna and skipjack tuna and found the levels of two metals have decreased throughout canning process. The same study also stated that defrosting, cooking and autoclaving decreased the Cd and Pb concentrations in the fish. The authors have argued that decreased metal concentrations during the thermal processes are due to reduced protein contents and also due to removing of heavy metals (as free salts) with leaching water. Further, the changes of the pH in the canning medium due to the accompanying liquids, also believed to cause alterations in the chemical forms of the metals.

Conclusion: The results showed no significant differences in heavy metal contents in green leafy vegetables, among raw, cooked and stir-fried methods ($p<0.05$). Therefore, the type of processing method has a minimal effect in reducing the heavy metal contents of green leafy vegetable samples. Further studies are necessary to investigate the bioaccessibility and toxicity of trace elements from green leafy vegetables and to establish suitable therapeutics measures for chronic and acute intoxication of dietary heavy metal intake.

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