Chemistry International 4(1) (2018) 15-23

International Scientific Organization
http://iscientific.org/
Chemistry International
www.bosaljournals.com/chemint/

Effect of cooking time on selected metals, oxalate and phytate contents of the raw and cooked lettuce from five farms in Ethiopia

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A R T I C L E I N F O

Article type:
Research article
Article history:
Received September 2017
Accepted January 2018
January 2018 Issue
Keywords:
Lettuce
Effect of cooking
Mineral composition
Anti-nutritional factors
Bioavailability
Chemical composition
Oxalate and phytate

A B S T R A C T

The effect of cooking time on mineral composition and anti-nutritional factors, oxalate and phytate, of lettuce grown in five farms of Ethiopia was investigated in this study by flame atomic absorption spectrometry, titrimetry, and spectrophotometrically, respectively. The work was focused in the evaluation of bioavailability of Ca, Fe and Zn by the molar ratios of [Phy]:[Fe], [Phy]:[Zn], [Ca]:[Phy], ([Ca][Phy])/[Zn]. The mineral composition was found to be: 1557–3171, Ca; 13.8–14.7, Mg; 0.7–2.8, Zn; 21.4–123, Fe; 3.94–9.41, Mn; 0.39–1.19, Cu; ND–0.24, Co; and 1.46–2.63, Ni; in mg/100 g in the raw samples. They all show decreasing on cooking except Fe, Zn and Ca which show significant increment depending on cooking time interval. The anti-nutritional factors in uncooked lettuce may present health-hazard potential, which in turn demands cooking for short time before consumption to eliminate the toxic effects of anti-nutritional factors.

Capsule Summary: The effect of cooking time on mineral composition and anti-nutritional factors, oxalate and phytate, of lettuce grown in five farms area of Ethiopia was investigated in this study by flame atomic absorption spectrometry, titrimetry, and spectrophotometrically, respectively.

Cite This Article As: A. Lakew, B. S. Chandravanshi, A. Belay and G. Behailu. Effect of cooking time on selected metals, oxalate and phytate contents of the raw and cooked lettuce from five farms in Ethiopia. Chemistry International 4(1) (2018) 15-23.

INTRODUCTION

Plant foods are the major staples of diets in developing countries like Ethiopia, in which the consumption of animal-source foods is often low because of economic and/or religious concerns. Vegetables constitute an important part of the human diet since they contain carbohydrates, proteins, as well as vitamins, minerals, and trace elements (Dastane, 1987).

Lettuce (*Lactuca sativa* L.) belongs to the Composite (sunflower or daisy family). It is an annual plant native to the Mediterranean area (Davey et al., 2007). Lettuce is reported to accumulate more of heavy metals which can be transferred to humans on its consumption (Intawongse and Dean, 2006).

All form of living matter requires many minerals for their life processes. Plant-based diets are often associated with micronutrient deficits, exacerbated in part by poor micronutrient bioavailability. The diet-related factors have a greater influence on the bioavailability of the micronutrients

Table 1: Recovery results of some of the minerals (Pb, Zn, Ni, Cd, Cr, and Cu)*

Mineral	Amount in lettuce (mg/100 g)	Amount added (mg/100 g)	Total expected (mg/100 g)	Amount assessed (mg/100 g)	Recovery value (%)
Pb	0.265	0.3	0.57	0.56	98
Cu	0.63	0.5	1.13	1.10	94
Zn	0.71	1.0	1.71	1.68	97
Ni	0.68	0.5	1.18	1.14	92
Cr	0.24	0.3	0.54	0.52	93
Cd	0.021	0.05	0.071	0.07	98

*The values are on dry weight basis.

in plant foods, particularly Ca, Fe and Zn, than on the macronutrients (Mohite et al. 2013; Ayele et al., 2015).

Phytate has been recognized as an anti-nutrient due to its adverse effects. It reduced the bioavailability of minerals and caused growth inhibition. It forms insoluble complexes with Cu^{2+}, Zn^{2+}, Fe^{3+} and Ca^{2+} and as a result reduces the bioavailability of these essential minerals. Especially Zn and Fe deficiencies were reported as a consequence of high phytate intake (Greiner and Konietzny, 2006; Greiner et al., 2006).

Phytate, zinc, and calcium contents of 30 East African foods and their phytate:Zn, Ca:phytate, and [Ca][phytate]/[Zn] molar ratios have been reported by Ferguson et al. (1988). The study also reported that cooking had no effect on the phytate content of cereals, but milling and fermentation reduced both the phytate and zinc contents of maize flour. Effect of local food processing on phytate levels in cassava, cocoyam, yam, maize, sorghum, rice, cowpea, and soybean has been reported by Marfo et al. (1990).

Oxalic acid forms an insoluble calcium salt with a 1:1 molar stoichiometry. The calcium oxalate may also precipitate around soft tissues such as the kidney, causing kidney stones (Umaru et al., 2007). Thus a fraction of dietary Ca is rendered unavailable for absorption. Soaking and cooking of foodstuffs high in oxalate reduce the oxalate content by leaching (Noonan and Savage, 1999). Boiling may cause considerable skin rupture and facilitate the leakage of soluble oxalate into cooking water (Bhandari and Kawabata, 2004; Poeydomenge and Savage, 2007).

Mosha et al. (1995) have reported the effect of blanching on the antinutritional content in cabbage, turnip, collard, sweet potato and peanut leaves. Levels of tannic acid and phytic acid were significantly reduced by conventional and microwave blanching methods while oxalic acid levels were not significantly reduced in most of the treatments by either of the blanching methods.

According to Yadav and Sehgal (2002), blanching of green leafy vegetables induces moderate losses of 5-15% of phytate in vegetables. Furthermore, it is desirable to establish optimum time and temperature to process food in order to derive maximum health benefits. Ayele et al. (2015) have reported the effect of cooking temperature on mineral content and antinutritional factors of yam and taro grown in southern Ethiopia. The study showed significant decrease in the phytate and oxalate contents of yam and taro on cooking and increase in the bioavailability of minerals in the cooked samples.

Recently some studies have been carried out on the mineral contents of plant-based food: kocho and bulla (Atlabachew and Chandravanshi, 2008), enset (Debebe et al., 2012), vegetables (Weldegebriel et al., 2012), cactus pear (Aregahegn et al., 2013a), yam (Aregahegn et al., 2013b), white lupin (Akalu and Chandravanshi 2014), linseed (Mekebo and Chandravanshi 2014) and ginger (Wagesho and Chandravanshi 2015) used in Ethiopia but not on the anti-nutritional factors and bioavailability of minerals except on yam and taro (Ayele et al., 2015).

The objectives of this research were to assess the cooking effect on minerals, oxalate and phytate in the lettuce cultivated in Ethiopia and to assess the inhibitory effect of oxalate and phytate and effect of cooking on the bioavailability of calcium, iron and zinc in lettuce produced with irrigated rivers of Akaki, Bulbula and Ziway Lake of Ethiopia. Therefore total content and bioavailability of metals, phytate and oxalate were determined in lettuce plants, before and after cooking.

MATERIAL AND METHODS

Instruments

Muffle furnace (Carbolite Astonlane, Hope, Sheffield, England), atomic absorption spectrophotometer (AA-6800 AAS Shimadzu, Japan), UV-Vis spectrophotometer (CECIL, CE 1021, 1000 series, UK), centrifuge (DYNAC II centrifuge, clay adams, division of Becton Dikinson and Company, USA), Ohaus Adventurer Analytical balance (USA), hotplate (Wagtech hot plate, UK), laboratory grinding mill (Kika-werke M 20, Germany), Stuart magnetic stirrer (Wagtech,

Table 2: The of ash, phytate, and oxalate contents for raw and cooked lettuce samples in (mg/100 g) (n = 3)*

Sample code	Phytate	Oxalate	% Ash
Akaki 0''	199±1.9	18.0±0.4	20.9±0.1
Akaki 30''	76.0±1.2	12.0±0.4	19.1±0.1
Akaki 60''	29.0±1.7	8.88±0.5	17.9±0.1
Akaki 90''	1.00±0.1	6.36±3.1	16.2±0.1
Akaki 120''	48.3±1.9	6.27±2.8	14.4±0.2
Peacock 0''	175±1.8	11.4±0.8	20.5±0.2
Peacock 30''	131±1.4	9.04±1.1	18.9±0.2
Peacock 60''	105±1.1	8.32±1.7	18.6±0.1
Peacock 90''	27.7±0.6	7.85±1.3	17.9±0.03
Peacock 120''	52.8±2.2	7.62±0.3	14.5±0.1
**Kera Br 0''	485±3.5	15.2±0.3	20.8±0.4
Kera Br 30''	182±6.3	9.09±0.3	17.9±0.03
Kera Br 60''	35.3±4.2	8.66±0.1	17.8±0.1
Kera Br 90''	20.8±1.5	5.13±0.4	16.2±0.1
Kera Br 120''	23.1±1.4	5.10±0.3	14.4±0.2
Ziway 0''	22.2±2.6	8.86±2.7	21.8±0.1
Ziway 30''	19.7±2.4	5.78±2.5	20.3±0.3
Ziway 60''	4.97±2.1	5.62±0.3	19.6±0.4
Ziway 90''	1.23±1.2	5.40±0.2	19.1±0.4
Ziway 120''	1.68±1.4	5.18±0.2	14.4±0.1
Sebeta 0''	13.7±1.7	10.6±0.7	20.3±0.3
Sebeta 30'	11.2±2.6	8.59±1.1	19.6±0.1
Sebeta 60''	5.69±4.9	5.54±1.9	19.3±0.2
Sebeta 90''	5.12±2.2	5.14±1.5	16.2±0.1
Sebeta 120''	7.36±6.3	4.95±3.2	14.5±0.1

*The values are on dry weight basis. **Kera, Br 0'' = brown colour lettuce sample from Kera. Sample code 0'' = uncooked or raw sample, sample code (30'', 60'', 90'', 120'') = cooking time intervals for 30, 60, 90, 120 s.

UK), flame photometer (Jenway PFP7, UK) and desiccator (SCN Simax, Germany) were used.

Chemicals

Analytical reagent grade chemicals were employed for the preparation of all solutions. From standard metal ion solutions (1000 mg/L) which were purchased from the AAS Company Vorna Valley (ICP AAS standard, Germany), the desired concentration prepared by dissolving appropriate amounts of the nitrates in double-distilled water and were diluted daily for obtaining reference and working solutions. Jenway flame photometer standards (1000 mg/L, UK) for Na and K, sulfosalisalic acid (Merck, Germany), $FeCl_3.6H_2O$ (Merck, Germany), sodium phytate salt (phytic acid dodeca sodium salt hydrate water 10–15% product, Aldrich, USA), concentrated ammonia (about 33% w/w AR, Eurostar Scientific Ltd, UK), $CaCl_2.2H_2O$ (Merck, Germany), H_2SO_4 (Riedel-DeHaen, Germany), potassium permanganate (Merck KGaA, 64271 Darmstadt, Germany), HCl (37% Riedel-Dehaen, Sigma-Aldrich Chemicals GmbH, Germany) and HNO_3 (about

Table 3: Mean mineral (Fe, Mg, K, Na, Ca, Zn, Cu) contents of lettuce with standard deviation (mg/100 g)*. Treated as raw and cooked at five different time intervals 0, 30, 60, 90,120 s at 93 ^{o}C (n = 3)

Sample code	Fe	Mg	K	Na	Ca	Zn	Cu
Akaki 0''	105±0.13	14.7±0.17	7253±1.1	1770±3.7	2315±4.6	1.05±0.03	0.39±0.0
Akaki 30''	233±0.19	13.6±0.15	7418±1.6	1502±4.3	2561±4.2	1.06±0.04	0.38±0.02
Akaki 60''	231±0.13	13.7±0.15	7651±2.3	1682±2.8	2555±6.9	1.09±0.15	0.34±0.1
Akaki 90''	144±0.07	13.4±0.16	7701±0.9	1685±4.1	2526±5.7	1.55±0.0	0.24±0.2
Akaki 120''	144±0.16	13.4±0.08	7158±3.9	1383±4.4	2683±3.7	1.54±0.22	0.23±0.2
Ziway 0''	123±0.24	13.8±0.14	7487±3.9	2387±4.3	3171±4.6	2.19±0.0	0.63±0.0
Ziway 30''	147±0.21	13.4±0.20	7634±2.8	2231±1.5	4225±5.8	2.19±0.03	0.63±0.01
Ziway 60''	147±0.25	13.4±0.12	7554±1.0	2382±3.3	4218±5.2	2.27±0.1	0.68±0.1
Ziway 90''	143±0.30	13.5±0.03	7194±4.2	2375±1.5	2539±2.4	2.55±0.3	0.67±0.07
Ziway 120''	105±0.12	13.1±0.07	6329±2.1	1383±4.4	2318±3.7	1.8±0.4	0.60±0.02
**Kera Br 0''	33.2±0.31	13.8±0.22	8164±2.4	1244±1.8	2052±1.1	0.7±0.01	1.19±0.1
Kera Br 30''	39.8±0.22	16.5±0.17	7717±2.2	1293±2.6	2565±4.9	0.71±0.2	0.87±0.02
Kera Br 60''	39.7±0.21	16.5±0.17	7443±3.5	1237±1.8	1956±2.5	1.38±0.02	0.82±0.06
Kera Br 90''	25.2±0.70	14.9±0.16	7684±3.5	1286±3.2	2550±4.3	1.45±0.02	0.65±0.01
Kera Br 120''	22.4±0.60	13.1±0.17	6329±2.1	1383±4.4	2319±4.6	1.4±0.5	0.63±0.0
Peacock 0''	21.4±0.14	14.4±0.11	7431±3.8	3101±1.2	1557±5.1	2.8±0.01	0.63±0.0
Peacock 30''	28.6±0.15	14.3±0.17	7422±1.1	3105±4.1	1539±2.6	2.76±0.02	0.63±0.03
Peacock 60''	28.2±0.16	14.7±0.14	7331±3.2	2962±3.7	1844±5.2	2.82±0.2	0.57±0.01
Peacock 90''	26.2±0.14	14.1±0.13	7578±3.2	3454±3.5	2309±4.2	2.82±0.1	0.54±0.02
Peacock120''	24.8±0.12	13.1±0.17	6329±2.1	1444±3.4	2319±4.6	2.86±0.1	0.47±0.08
Sabeta 0''	35.3±0.13	14.7±0.19	8045±3.9	803±3.7	3043±5.6	2.48±0.6	0.95±0.1
Sebeta 30''	46.1±0.08	16.9±0.13	8875±4.0	862±5.1	3379±5.8	2.34±0.5	0.88±0.1
Sebeta 60''	46.7±0.87	15.2±0.14	8913±3.4	1394±2.4	3075±4.1	2.6±0.3	0.50±0.2
Sebeta 90''	45.5±0.12	15.5±0.15	8811±1.8	915±4.3	2748±6.6	2.39±0.2	0.53±0.2
Sebeta 120''	39.7±0.15	11.6±0.15	6317±4.0	891±4.1	2315±5.6	2.24±0.8	0.51±0.02

*The values are on dry weight basis. **Kera Br 0'' = brown colour lettuce sample from Kera. Sample code 0'' = uncooked or raw sample. Sample code (30'', 60'', 90'', 120'') = cooking time intervals for 30, 60, 90, 120 s. ND = not detected (below the detection limit)

69% LR, Eurostar Scientific Ltd., UK) were used as received. Freshly prepared distilled deionized water was used in all experiments.

Plant sampling, preparation and analysis

To meet the objectives set, from a total of five farms Akaki, Kera, Peacock, Ziway and Sebeta, recently matured leaves of lettuce (*Lectuca sativa*) samples were handpicked then labelled and brought in plastic bags to Ethiopian Public Health Institute. The samples were immediately washed by distilled water and subsequently rinsed with deionized water to eliminate all contaminants including air borne pollutants. The samples were cooked/boiled at 93 ^{o}C (water boils at 93 ^{o}C in Addis Ababa due to its high altitude) at five different

Table 4: Mean mineral (Pb, Co, Cr, Mn, Ni, Cd) contents of lettuce with standard deviation (mg/100 g)*. Treated as raw and boiled at five different boiling time intervals 0, 30, 60, 90,120 s at 93 ^{o}C (n = 3)

Sample code	Pb	Co	Cr	Mn	Ni	Cd
Akaki 0''	0.198±0.01	0.24±0.02	4.69±0.04	9.41±0.08	2.34±0.15	0.021±0.01
Akaki 30''	0.194±0.01	0.16±0.01	4.49±0.22	9.06±0.8	2.23±0.16	0.018±0.01
Akaki 60''	0.188±0.01	0.011±0.0	4.48±0.22	9.03±0.08	2.22±0.16	0.015±0.01
Akaki 90''	0.159±0.02	0.009±0.0	1.11±0.08	5.8±0.05	0.95±0.02	0.011±0.01
Akaki 120''	ND	0.001±0.0	0.11±0.01	4.79±0.05	0.24±0.08	ND
Ziway 0''	0.149±0.01	ND	1.43±0.02	6.97±0.05	1.98±0.05	0.020±0.01
Ziway 30''	0.143±0.01	ND	1.39±0.31	5.49±0.05	1.93±0.12	0.019±0.0
Ziway 60''	0.126±0.02	ND	1.38±0.29	5.42±0.03	1.80±0.01	0.019±0.0
Ziway 90''	0.045±0.01	ND	1.35±0.21	5.24±0.01	1.10±0.11	0.017±0.0
Ziway 120''	0.040±0.01	ND	1.29±0.21	5.17±0.01	1.09±0.10	0.017±0.0
**Kera Br 0''	0.265±0.03	0.24±0.01	2.64±0.12	4.56±0.14	2.63±0.06	0.02±0.01
Kera Br 30''	0.230±0.03	0.193±0.0	2.56±0.28	4.51±0.04	2.62±0.14	0.016±0.0
Kera Br 60''	0.228±0.03	0.178±0.0	2.48±0.02	4.42±0.04	2.43±0.08	0.014±0.02
Kera Br 90''	0.221±0.03	ND	1.61±0.13	4.06±0.06	1.35±0.01	0.013±0.0
Kera Br 120''	0.213±0.03	ND	1.29±0.21	3.96±0.05	1.09±0.10	0.013±0.02
Pecokeck 0''	0.071±0.02	ND	1.29±0.03	4.02±0.06	1.76±0.05	0.002±0.01
Peacock 30''	0.069±0.02	ND	1.19±0.04	3.99±0.05	1.75±0.05	0.002±0.01
Peacock 60''	0.057±0.01	ND	1.14±0.31	4.01±0.01	1.23±0.07	0.003±0.0
Peacock 90''	0.053±0.02	ND	1.13±0.01	4.01±0.3	1.22±0.08	0.002±0.01
Peacock 120''	0.024±0.03	ND	1.09±0.21	3.52±0.04	1.19±0.10	0.001±0.01
Sebeta 0''	ND	ND	2.3±0.07	4.94±0.04	1.46±0.25	0.002±0.0
Sebeta30''	ND	ND	2.16±0.05	4.11±0.09	1.42±0.14	0.001±0.0
Sebeta 60''	ND	ND	2.13±0.06	4.16±0.05	1.37±0.06	0.001±0.0
Sebeta 90''	ND	ND	2.12±0.3	3.51±0.02	1.30±0.16	0.001±0.0
Sebeta 120''	ND	ND	1.11±0.21	3.17±0.01	1.19±0.03	0.001±0.0

*The values are on dry weight basis. **Kera Br 0'' = brown colour lettuce sample from Kera. Sample code 0'' = uncooked or raw sample. Sample code (30'', 60'', 90'', 120'') = cooking time intervals for 30, 60, 90, 120 s. ND = not detected (below the detection limit).

time intervals (raw or 0, 30, 60, 90, 120 s) then the water drained off. After treatment the hot samples were exposed to the air to allow surface water to evaporate. Immediate browning of the pieces was observed after boiling that may be due to enzymatic browning by polyphenol oxidase.

All the raw and cooked samples were dried in an oven at 50 ^{o}C for overnight until it gets constant weight and ground to fine powder by using a laboratory grinding mill and sieved through a mesh size of 1 mm in diameter. Samples from each location were taken and analyzed in triplicate (n = 3).

Each of the five sets homogenized lettuce samples (raw and cooked at 30, 60, 90 and 120 s at 93 ^{o}C) were analyzed for their oxalate, phytate, and minerals (Ca, K, Na, Mg, Fe, Zn,

Table 5: Values of the [Phy]:[Fe], [Phy]:[Zn], [Ca]:[Phy], [Ox]:[Ca] and ([Ca][Phy])/[Zn] molar ratios of lettuce samples*

Sample code	[Ca]:[Phy] molar ratio	[Phy]:[Zn] molar ratio	[Phy]:[Fe] molar ratio	[Ox]:[Ca] molar ratio	([Ca][Phy])/ [Zn]
Akaki 0''	192	18.8	0.16	0.004	1089
Akaki 30''	552	7.84	0.03	0.002	498
Akaki 60''	1415	2.68	0.01	0.002	170
Akaki 90''	3789	0.70	0.01	0.001	44.4
Akaki 120''	907	3.11	0.03	0.001	206
Pecokeck 0''	297	7.92	0.12	0.002	625
Peacock 30''	531	5.92	0.08	0.001	623
Peacock 60''	658	4.60	0.06	0.001	483
Peacock 90''	1511	1.08	0.02	0.001	68.2
Peacock120''	723	2.91	0.04	0.001	168
Kera Br 0''	70.3	68.6	1.24	0.003	3542
Kera Br 30''	233	25.4	0.39	0.002	1627
Kera Br 60''	913	2.53	0.07	0.002	124
Kera Br 90''	2019	1.42	0.07	0.001	90.8
Kera Br 120''	1652	1.64	0.09	0.001	94.7
Ziway 0''	1172	0.79	0.09	0.003	31.0
Ziway 30''	1296	0.71	0.08	0.002	27.3
Ziway 60''	6148	0.17	0.02	0.001	8.09
Ziway 90''	30861	0.04	0.0004	0.001	2.13
Ziway 120''	22736	0.06	0.0001	0.001	3.37
Sebeta 0''	3691	0.55	0.03	0.002	42.2
Sebeta 30'	4937	0.26	0.01	0.001	21.5
Sebeta 60''	8849	0.22	0.01	0.001	16.5
Sebeta 90''	8737	0.09	0.01	0.001	6.38
Sebeta 120'	5189	0.28	0.02	0.001	16.0

*The values are on dry weight basis.

Fe, Co, Cu, Mn, Ni, Cd and Pb). The ash content was determined using muffle furnace, by the methods developed with Association of Official Analytical Chemists (AOAC) (2000). The ashes were digested by HCl (37%) and the mineral content was determined by FAAS using air-acetylene flame (Mertz, 1981). The phytate content of raw and cooked lettuce samples was determined by Latta and Eskin (1980) method using Wade reagent (1:1 ratio of 0.03% solution of $FeCl_3 \cdot 6H_2O$ containing 0.3% sulfosalicylic acid in water). The absorbance at 500 nm was measured using UV-Vis spectrophotometer. The oxalate contents were determined using the method of Iwuoha and Kalu (1994). After the determination of phytate and oxalate, the molar ratio of phytate and oxalate to calcium, zinc and iron were calculated to evaluate the effect of elevated levels of phytate and oxalate on the bioavailability of dietary minerals (Omoruyi et al., 2007). Samples from each farm was taken and analyzed in triplicate (n = 3). The data obtained were subjected to multiple comparison tests using SPSS package, version 20. Mean separations were calculated by the general linear model procedures; post Hoc (LSD) multiple range tests with probability, $p < 0.05$. Statistical difference between the raw and cooked food was established using analysis of paired-samples t-test. All the results for minerals, oxalate and phytate were reported as mean value with their respective standard deviations.

RESULTS AND DISCUSSION

Validation of accuracy

A raw sample whose minerals, phytate and oxalate was determined was taken and known concentration of standard minerals, phytate and oxalate were added to them with the purpose of providing validation of accuracy of the procedure

used. The spiked samples were then analyzed and the results of the samples containing the added amount of standards were compared to the expected increase in the parameter to be analyzed relative the control (Fruhbeck et al., 1995). The results of recovery of minerals are given in Table 1. The recoveries of phytate and oxalate were found to be 94% and 98%, respectively. The percentage recoveries of the minerals and antinutrients from the samples were found between 92% and 98%, which are within the acceptable range (Hernandez-Mendoza et al., 2013).

Effect of cooking time on phytate and oxalate

Different cooking times studied have varied effect in reducing the levels of phytate and oxalate in lettuce. Phytate and oxalate shows considerable loss during cooking of lettuce. The effect shows significant difference within treatments (different cooking time intervals) at $p < 0.05$. The reduction ranges of phytate on cooking were 19–24% and 35–60% for oxalate in lettuce. The reduction of these anti-nutrients levels on cooking expected to enhance the mineral content of the lettuce, if the relative rate of migration of minerals during cooking is less. This study indicated that the studied anti-nutritional factors, though showing a high concentration in raw lettuce, will not pose a problem in human consumption if the lettuces are properly processed. Consumption of such properly cooked vegetable may serve as an additional dietary source for the alleviation of malnutrition.

The percentage ash of the sample gives an idea about the inorganic content of the samples from where the mineral content could be obtained. The ash content of the cooked samples indicates that the effect of cooking on the ash content of lettuce samples is markedly related with the cooking time. The results (Table 2) show that the mean ash content of the raw sample is significantly different at ($p < 0.05$) from the cooked ones. This may be due to leaching of soluble minerals (Na and K leached during cooking) (Lewu et al., 2009).

The mineral contents of raw and cooked lettuce samples are given in Table 3 and 4. The effect of cooking on the calcium content did not show significant difference ($p < 0.05$) between raw and cooked lettuce at all the four time intervals. The Ca:phytate molar ratio in all cases are greater than 6:1 (Table 5), that shows the good bioavailability of Ca (Wise, 1983). The mean Zn content shows significant difference ($p < 0.05$) between the raw and all the cooked samples for four cooking time intervals. Cooking to the three time intervals, 30, 60 and 90 s show an increasing concentration of Zn but it decreases at 120 s for all the samples, this may be because of leaching of the minerals during cooking. Similarly Phy:Zn ratio ranges from 0.55–7.92 mg/100 g, except for the brown lettuce with 68.6 mg/100 g, and for Akaki sample 18.8 mg/100 g (Table 5). Phy:Zn molar ratio shows the relative increment of the denominator (the mineral) as it is reported (Mark et al., 2000). For the lesser bioavailability of Zn to happen Phy:Zn molar ratio has to be greater or within 10–15 (Lestienne et al., 2005). But no values fell in this range so that phytate induced Zn deficiency is not expected.

The phytate/iron molar ratios are used to predict the inhibitory effect on the bioavailability of minerals. A phytate/iron molar ratio > 1 is regarded as indicative of poor iron bioavailability (Ma et al., 2005). The mean Fe content shows significant difference ($p < 0.05$) between the raw and all the four cooking time intervals. The content of iron shows increment during cooking for the three time intervals, 30, 60 and 90 s but it decreases at 120 s. This may be due to the oxidation state of iron, i.e. Fe^{+3} is less soluble than Fe^{+2}, however, this needs a further study for in which oxidation state does Fe exists in the lettuce samples. The molar ratio of phy:Fe (Table 5) is less than 0.16 which is indication of the bioavailability. In all the cooking time intervals except the Kera brown lettuce with phytate:Fe molar ratio 1.24, though it shows progress on cooking for most of the samples.

Except manganese the effect of cooking on the mean sodium, potassium, magnesium and other trace metal content did not shows significant difference at the $p < 0.05$ level between raw and cooked lettuce.

CONCLUSIONS

This study found that lettuce contains significant amounts of major and trace minerals. The cooking/boiling times at 60 and 90 s of lettuce is the optimum time to get a significant difference in the samples for the bioavailability of minerals. At 30 s no significant difference due to insufficient time to break the cell wall and to release the minerals. At 120 s, the metals concentration decreases due to the total loss of minerals because the cell wall is broken down further to occur dissolution to the water and discarded, though the bioavailability decreases. Therefore the lettuce must be cooked/boiled between 60 to 90 s (1:00–1:30 min) at the boiling temperature of water, i.e. 93 ^{o}C to effectively decrease the phytate and oxalate content and to deserve the minerals of lettuce. In general the study results indicated that lettuce is good sources of minerals with high predicted bioavailability for Ca, Fe and Zn if processed properly, i.e. 93 ^{o}C, for 60 to 90 s, not to miss the minerals drained with the boiling water.

ACKNOWLEDGEMENTS

The authors acknowledge the financial and material support made by the Department of Chemistry of Addis Ababa University, Ethiopia, and the Ethiopian Public Health Institute, Addis Ababa, Ethiopia, for providing the laboratory facilities.

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