

## Examination of the Lemon Effect on Risk Elements Concentrations in Herbal and Fruit Teas

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### Abstract

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Fennel, mint (peppermint), and sage herbal teas and apple, lemon, and rosehip fruit teas were selected for the determination of the following risk elements contents: aluminium (Al), arsenic (As), barium (Ba), cadmium (Cd), nickel (Ni), lead (Pb), and antimony (Sb). Moreover, the effect of lemon on these elements contents was also examined. Inductively Coupled Plasma Optical Emission Spectrometry (ICP-OES) was used for these experiments on selected teas (2 g of tea infused in 100 ml of water). The maximum changes of elements concentrations after the lemon addition were as follows: Al 1077 µg/l in lemon tea; Ba 12560 µg/l in rosehip tea; Cd 183 µg/l in sage tea; Ni 1136 µg/l in fennel tea; and Pb 238 µg/l in lemon tea. Both As and Sb were below the detection limits in pure tea and lemon-infused teas. This study indicated that after the lemon addition, rosehip tea had a hazard index (HI) value of  $10827 \times 10^{-4}$  for 200 ml/day (2 cups/day), which represents a high risk for human health. If lemon is added to rosehip tea for consumption, 100 ml/day is recommended according to the calculated HI values.

**Keywords:** Hazardous elements; tea infusion; human health; ICP-OES; HI

Turkey is an important country in terms of tea production with a share of 4%. In 2011, Turkey ranked fifth in the world tea production after China, India, Kenya, and Sri Lanka according to the statistics of the Food and Agriculture Organisation (FAO) of the United Nations (FAO 2013). The teas most preferred by Turkish society after black tea (*Camellia sinensis*) are fennel (*F. vulgare* ssp. *piperitum*), peppermint (*Mentha piperita* L.), and sage (*Salvia officinalis* L.) herbal teas, as well as apple (*Malus domestica* Borkh), lemon (*C. limon*), and rosehip (*Rosa canina* L.) fruit teas.

Fennel is highly recommended for diabetes, bronchitis, chronic coughs, and kidney stones, and it is considered to have diuretic, stomachic, and galactagogue properties (BARROS *et al.* 2010; RAWSON *et al.* 2013). Peppermint tea is a popular single in-

gredient herbal tea and is recommended for biliary disorders, dyspepsia, enteritis, flatulence, gastritis, and intestinal colic, in addition to spasms of the bile duct, gallbladder, and gastrointestinal (GI) tract (McKAY & BLUMBERG 2006). The main widely used therapeutic effect of common sage as tea is a soothing and antiseptic effect on mucus (PATENKOVIC *et al.* 2009; VERMAAKA *et al.* 2009). Apple tea (apple peel extract) is an effective inhibitor of the angiotensin converting enzyme (ACE), which is a major causative factor in the development of hypertension (BALASURIYA & RUPASINGHE 2012). Bioactive components in lemon tea (lemon seed extracts) can be a good source of antioxidants and can induce apoptosis in human breast cancer cells (MCF-7 cells) (KIM *et al.* 2012). The aggregate fruit of rosehip is used for the

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prevention of diseases in the form of rosehip tea consumed during periods with increased risks of the common cold (SZENTMIHALYI *et al.* 2002).

Herbal and fruit teas should be consumed cautiously because of their content of risk elements (Al, As, Ba, Cd, Ni, Pb, and Sb) that may cause negative effects on the human body.

According to previous investigations, the exposure to Al may cause encephalopathy/dialysis dementia, Parkinson's disease, and Alzheimer's disease (LOPEZ *et al.* 2002; JALBANI *et al.* 2007). The As level in tea infusions and the leaching characteristics of As in water are more closely associated with consumer health than the original tea leaf (YUAN *et al.* 2007). Barium toxicity may cause muscular paralysis and gastrointestinal disturbances (MILLOUR *et al.* 2012). Cadmium exposure may affect adverse health effects, including kidney damage and possibly also bone fractures (CAO *et al.* 2010; OLAWOYIN *et al.* 2012). Among the most sensitive endpoints resulting from oral exposure of Ni is exacerbation of existing dermatitis in Ni-sensitive individuals (BROUWERE *et al.* 2012). Lead exposure may lead to memory deterioration, prolonged reaction times, and reduced comprehension ability (CAO *et al.* 2010). Recent studies have shown that Sb pollution is a global issue because of its toxicity in causing diseases of the liver, skin, respiratory system, and cardiovascular system (WU *et al.* 2011).

Toxic contamination by risk elements, including Pb, Al, Cd, and As, has been found in most of the teas sampled in the region of China, India, and Sri-Lanka (SCHWALFENBERG *et al.* 2013). Studies by YOKEL and FLORENCE (2008) suggest that tea can provide a significant amount of Al for systemic circulation,

which can cause toxicity to target organs. A small sample size from Iranian and other unknown foreign country samples released up to 1.093 mg/l of Cd into the tea infusion, which is considered hazardous, as it exceeds the provisional tolerable weekly intake (PTWI) guidelines from tea drinking alone (SHOKRZADEH *et al.* 2008). NOOKABKAEW *et al.* (2006) stated that the daily intake of all elements from herbal tea (*Gynostemma pentaphyllum*, *Camellia sinensis*, and *Morus alba*) infusions (three cups/day) is within the average daily intake. Therefore, herbal teas need not produce any health risks for human consumption if other sources of toxic-metal-contaminated food are not consumed at the same time.

The element contents of herbal and fruit infusions have been previously studied (Table 1) as follows: OZCAN and AKBULUT (2007) used Inductive Coupled Plasma Optical Emission Spectrometry (ICP-OES) to evaluate the Al, As, Ba, Cd, Ni, and Pb contents of fennel, rosehip, and sage infusions; MALIK *et al.* (2013) studied the Al and Ni contents of rosehip infusions using ICP-OES; PYTLAKOWSKA *et al.* (2012) analysed the Al and Ba contents of sage infusions using ICP-OES; and PYTLAKOWSKA *et al.* (2012) and CINDRIC *et al.* (2013) analysed the Al, Ni, and Pb contents of sage infusions using ICP-OES. The following studies have reported the risk elements contents of mint infusions: LOZAK *et al.* (2002) analysed the As, Ba, Cd, Ni, and Pb contents of peppermint-leaf infusions using Inductively Coupled Plasma Mass Spectrometry (ICP-MS); and SALVADOR *et al.* (2002) analysed the Ni contents of mint infusions using the Energy-Dispersive X-Ray Fluorescence (EDXRF) technique. SALVADOR *et al.* (2002) also evaluated the Ni content of apple

Table 1. Published studies on the risk element concentrations (µg/l) of fennel, mint, sage, apple, lemon, and rosehip teas

Tea	Al	As	Ba	Cd	Ni	Pb	Reference
Fennel	0.9	0.79	0.23	0.001	0.9	0.14	OZCAN & AKBULUT (2007)
Mint		48 ± 4	758 ± 33	8 ± 1	2 580 ± 140 360–1 410	1 120 ± 140	LOZAK <i>et al.</i> (2002) SALVADOR <i>et al.</i> (2002)
	17 600 ± 1 000		3 580 ± 60				PYTLAKOWSKA <i>et al.</i> (2012)
Sage	< 0.22				0.26	2.99	CINDRIC <i>et al.</i> (2013)
	1.4	0.21	1.3	0.04	0.3	0.28	OZCAN & AKBULUT (2007)
Apple	2 900–4 300		16–9		90–170		FERNANDEZ <i>et al.</i> (2002) SALVADOR <i>et al.</i> (2002)
Lemon	2 900–4 300		16–9	300	3 300	nd	OTHMAN <i>et al.</i> (2012) FERNANDEZ <i>et al.</i> (2002)
Rosehip	183 ± 32 1.5	0.99	0.39	< 0.001	50 ± 15 0.2	0.17	MALIK <i>et al.</i> (2013) OZCAN & AKBULUT (2007)

nd – not detected

tea, and FERNANDEZ *et al.* (2002) used ICP-OES to analyse the Al and Ba contents of apple and lemon teas. The Cd, Ni, and Pb contents of lemon tea were evaluated using Flame Atomic Absorption Spectrometry (FAAS) by OTHMAN *et al.* (2012).

In this study, the risk-elements contents (Al, As, Ba, Cd, Ni, Pb, and Sb) of herbal teas (fennel, mint, and sage) and fruit teas (apple, lemon, and rosehip) were determined. In some countries, teas are consumed with lemon. Based on this consumption, which accounts for the novelty of our study, the risk-elements contents of the aforementioned teas were determined after the addition of lemon. ICP-OES was used for the analyses in this study. According to the daily consumed quantity and analysis results, the daily intake percentages of these elements were reported, and health risk assessment was performed for these risk elements contents in the selected teas.

## MATERIAL AND METHODS

**Sample preparation.** The selected teas (fennel, mint, sage, apple, lemon, and rosehip) and lemons were purchased from a Turkish market in Istanbul. The non-essential elements (Al, As, Ba, Cd, Ni, Pb, Sb, and Ti) in the selected tea infusions with and without lemon, which were prepared according to ISO-3103:1980 standard, had been determined by ICP-OES. Distilled water with lemon (1 : 6 ratio of lemon/pure water) was added to the infusions 15 min before the analysis. The pH of the individual infusions was measured by a Hanna Instruments Basic pH/ORP Benchtop Meter (Hanna Instruments, Michigan, USA).

**ICP conditions.** An ICP-OES (Perkin Elmer, Optima 2100) equipped with an AS-93 autosampler was used in the experiments. The instrument operating parameters are listed in Table 2.

The calibration solutions were prepared from standard certified elemental solutions (VHG Labs,

Table 2. Instrument operating conditions

Conditions	Values
Power	$1.45 \times 10^3$ W
Plasma gas flow rate	15 l/min
Auxiliary gas flow rate	0.8 l/min
Nebulizer type	cross flow
Nebulizer pressure	$117 \times 10^3$ Pa
Spray chamber type	scott type
Sample flow rate	1.5 l/min
Observation height	$15 \times 10^{-3}$ m (axial); 0 m (radial)
Integration time	min 1 s; max 5 s

Manchester, USA) and a 0.3% HNO<sub>3</sub> solution to obtain a range of concentrations. The linearity within the working range was verified. The correlation coefficient ( $R^2$ ) was  $> 0.999$  for all elements. Six replicates were performed, and the mean values were used to report the elements concentrations.

**Equations.** The daily consumption quantity of the selected herbal and fruit teas was accepted as two cups of tea (200 ml daily). The risk elements intakes from the herbal and fruit tea infusions with and without lemon in the human body were calculated using Eq. (1) as follows:

$$m \text{ (}\mu\text{g/day)} = C \text{ (}\mu\text{g/l)} \times 200 \text{ (ml/day)} \quad (1)$$

where:  $m$  – element content in two daily cups of tea;  $C$  – element concentration

The daily tolerated maximum (DTM) values of risk elements for the human body (males as 80 kg; females as 60 kg) were as follows: Al up to 11.43 mg/day for males and 8.57 mg/day for females (EFSA 2008); Ba up to 2 mg/day for males and 1.5 mg/day for females (US EPA 2005); Cd up to 0.08 mg/day for males and 0.06 mg/day for females; Ni up to 1 mg/day for both males and females (KARAK & BHAGAT 2010); Pb up to 0.57 mg/day for males and 0.43 mg/day for females (IPCS 2000); and Sb up to 0.069 mg/day for males and 0.051 mg/day for females (CHEUNG *et al.* 2008). Additionally, the daily intake percentages (DIPs) (%) of the risk elements were calculated using Eq. 2 as follows:

$$\text{DIP (\%)} = (m \times 100)/\text{DTM} \quad (2)$$

The health risk for As, Ba, Cd, Ni, Pb, and Sb contents due to the consumption of the teas studied was estimated in this study by the hazard index (HI) with Equation (3)–(5) (US EPA 2004; SHEN & CHEN 2008) as follows:

$$\text{Exposure dose} = C_i \times D_x \times E_d/B_w \times A_t \quad (3)$$

$$\text{Hazard quotient} = \text{Exposure dose/Reference dose (RfD)} \quad (4)$$

$$\text{Hazard index} = \sum_{n=1}^{n=k} \text{Hazard quotient} \quad (5)$$

where: RfD (mg/kg/day) values –  $3 \times 10^{-4}$  for As,  $2 \times 10^{-1}$  for Ba,  $1 \times 10^{-3}$  for Cd,  $2 \times 10^{-2}$  for Ni,  $1.43 \times 10^{-3}$  for Pb,  $4 \times 10^{-4}$  for Sb (US EPA 2004);  $C_i$  – element concentration in the infusion (mg/100 ml);  $D_v$  – daily consumption (200 ml/day);  $E_d$  – average exposure duration (50 years);  $B_w$  – average weight (70 kg);  $A_t$  – average lifetime (70 years)

Table 3. The concentrations of risk elements in selected Turkish herbal and fruit tea infusions with and without lemon addition

Method	Element (µg/l)				
	Al	Ba	Cd	Ni	Pb
Wavelength (nm)	396.153	233.527	228.802	231.604	220.353
LOD	2.0	1.8	0.3	0.8	1.8
MDL	6.0	4.0	1.0	2.0	2.0
LOQ	6.9	5.3	1.2	2.8	6.0
Fennel tea	I	nd	nd	9 ± 0.7	95 ± 7
	L	181 ± 11	117 ± 9	1 145 ± 98	299 ± 28
Mint tea	I	nd	4.4 ± 0.1	nd	nd
	L	nd	4.4 ± 0.1	2.4 ± 0.1	7.5 ± 0.7
Sage tea	I	1 283 ± 99	1 742 ± 146	421 ± 37	24 ± 2
	L	741 ± 69	1 801 ± 143	291 ± 27	220 ± 1
Apple tea	I	2 214 ± 189	183 ± 11	5.4 ± 0.1	191 ± 18
	L	2 627 ± 8	7 076 ± 263	489 ± 17	279 ± 27
Lemon tea	I	384 ± 33	6 238 ± 513	nd	250 ± 13
	L	1 461 ± 81	13 900 ± 1 336	59 ± 4	488 ± 34
Rosehip tea	I	1 942 ± 24	290 ± 9	7.4 ± 0.1	216 ± 16
	L	1 883 ± 74	12 850 ± 962	599 ± 11	378 ± 28

LOD – limit of detection; MLD – method detection limit; LOQ – limit of quantitation; I – infusions; L – lemon added infusions; nd –not detected

## RESULTS AND DISCUSSION

The concentrations of risk elements (Al, As, Ba, Cd, Ni, Pb, and Sb) in Turkish herbal tea (fennel, mint, and sage) and fruit tea (apple, lemon, and rosehip) infusions with and without lemon addition are shown in Table 3.

Differences occurred between the previously published results and the results of the present study

due to the region, soil, and climatic conditions of the growing tea. However, FERNANDEZ *et al.* (2002) reported that the Al contents of apple tea are between 2 900 and 4 300 µg/l, while this value was 2 214 µg/l in the present study (Tables 1 and 3).

The maximum values among the risk elements varied according to the tea type and method of consumption (with or without lemon) as follows: Ba

Table 4. Daily intake of risk elements in 2 cups of selected Turkish herbal and fruit tea infusions with and without lemon addition

Tea types	Method	Elements (µg)				
		Al	Ba	Cd	Ni	Pb
Fennel	I	–	–	–	1.8 ± 0.2	19.0 ± 1.4
	L	36.2 ± 2.2	23.4 ± 1.8	31.4 ± 0.6	229.0 ± 19.6	59.8 ± 5.6
Mint	I	–	8.8 ± 0.2	–	–	–
	L	–	8.8 ± 0.2	–	0.5 ± 0.02	1.5 ± 0.14
Sage	I	256.6 ± 19.8	348.4 ± 29.2	–	84.2 ± 7.4	4.8 ± 0.4
	L	148.2 ± 13.8	360.2 ± 28.6	36.6 ± 3.4	58.2 ± 5.4	44.0 ± 0.2
Apple	I	442.8 ± 37.8	36.6 ± 2.2	–	1.1 ± 0.02	38.2 ± 3.6
	L	525.4 ± 1.6	1 415.2 ± 52.6	33.4 ± 0.2	97.8 ± 3.4	55.8 ± 5.4
Lemon	I	76.8 ± 6.6	1 247.6 ± 102.6	–	–	50.0 ± 2.6
	L	292.2 ± 16.2	2 779.0 ± 267.2	11.8 ± 0.8	57.6 ± 5.4	97.6 ± 6.8
Rosehip	I	388.4 ± 4.8	58.0 ± 1.8	–	1.5 ± 0.02	43.2 ± 3.2
	L	376.6 ± 14.8	2 570.0 ± 192.4	34.4 ± 2.6	119.8 ± 2.2	75.6 ± 5.6

I – infusions; L – lemon added infusions

Table 5. Daily intake percentages of risk elements from 2 cups of selected Turkish herbal and fruit tea infusions with and without lemon addition

Tea types	Method	Elements (%)				
		Al	Ba	Cd	Ni	Pb
Fennel	I	–	–	–	0.18	3.33/(4.43)*
	L	0.32/(0.42)*	1.17/(1.56)*	39.25/(52.33)*	22.90	10.47/(13.95)*
Mint	I	–	0.04/(0.06)*	–	–	–
	L	–	0.04/(0.06)*	–	0.05	0.26/(0.35)*
Sage	I	2.25/(2.99)*	17.42/(23.23)*	–	8.42	0.84/(1.12)*
	L	1.30/(1.73)*	18.01/(24.01)*	45.75/(61.00)*	5.82	7.70/(10.27)*
Apple	I	3.88/(5.17)*	1.83/(2.44)*	–	0.11	6.69/(8.91)*
	L	4.60/(6.13)*	70.76/(94.35)*	41.75/(55.67)*	9.78	9.77/(13.02)*
Lemon	I	0.67/(0.90)*	62.38/(83.17)*	–	–	8.75/(11.67)*
	L	2.56/(3.41)*	139.00/(185.30)*	14.75/(19.67)*	5.76	17.08/(22.77)*
Rosehip	I	3.40/(4.53)*	2.90/(3.87)*	–	0.15	7.56/(10.08)*
	L	3.30/(4.39)*	129.00/(171.30)*	43.00/(57.33)*	11.98	13.23/(17.64)*

I – infusions; L – lemon added infusions; \*female intake percentages

(maximum of 13 900 µg/l) in lemon tea with lemon; Al (maximum of 2 627 µg/l) in apple tea with lemon; Ni (maximum of 1 145 µg/l) in fennel tea with lemon; Pb (maximum of 488 µg/l) in lemon tea with lemon; and Cd (maximum of 183 µg/l) in sage tea with lemon.

The effect of lemon on the selected teas resulted in changes in the risk-elements concentrations. After the addition of lemon, the following changes in the risk elements concentrations occurred: Al increased in fennel, apple, and lemon teas but decreased in sage and rosehip teas; Ba and Cd increased in all teas, except mint tea; Ni increased in fennel, mint, apple, lemon, and rosehip teas but decreased in sage tea; and Pb increased in all of the selected teas. The As and Sb contents in all the analysed teas with and without lemon were below the detection limit.

The measured pH values showed that the lemon addition changed the pH of tea. The pH values of the distilled water (28°C), lemon (25.2°C), and distilled water with lemon (1 : 6 ratio of lemon/pure water; 25.3°C) were 5.80, 3.41, and 3.46, respectively. The changes of pH in the analysed teas after the lemon addition were as follows: decreased from 6.30 (27.7°C) to 3.47 (25.2°C) in fennel tea; decreased from 6.10 (28.8°C) to 3.50 (25.2°C) in mint tea; decreased from 5.85 (28.1°C) to 3.44 (25.2°C) in sage tea; increased from 3.15 (27.4°C) to 3.41 (24.9°C) in apple tea; decreased from 5.64 (27.2°C) to 3.56 (25.2°C) in lemon tea; and increased from 3.39 (28.7°C) to 3.43 (25.1°C) in rosehip tea. Thus, the lemon addition

decreased the pH of the selected teas, except for the apple and rosehip teas. The acidic medium changed the elements contents of the teas.

The risk elements intakes from the herbal and fruit tea infusions with and without lemon in the human body are listed in Table 4. Using these values, the daily intake percentages (DIPs) (%) of the risk elements were calculated and are shown in Table 5.

The DIP ranges of the risk elements were as follows: the DIP of Al was below 7.00% in all selected teas; the DIP of Ba was between 0.04 and 4.00% in fennel tea with lemon, mint tea without and with lemon, as well as in apple and rosehip teas without lemon; the DIP of Ba was between 17.00% and 186.00% in sage and lemon teas without and with lemon as well as in apple and rosehip teas with lemon; and the DIP of Cd was between 14.00% and 61.00% in lemon, fennel, apple, rosehip and sage teas with lemon. The maximum DIP of Ni was 22.90% in fennel tea with lemon, and the maximum DIP of Pb was 22.77% in lemon tea with lemon.

HI values  $\geq 1.0$  represent unacceptable or high risk for health depending on the US EPA guidelines. The HI values of all the selected teas after lemon addition were close to 1.0 or exceeded 1.0, as shown in Table 6. The HI values of the teas with lemon were as follows: rosehip ( $10\,827 \times 10^{-4}$ ), lemon ( $9\,880 \times 10^{-4}$ ), fennel ( $8\,652 \times 10^{-4}$ ), apple ( $8\,611 \times 10^{-4}$ ), sage ( $7\,355 \times 10^{-4}$ ) and mint ( $110 \times 10^{-4}$ ). The increase of HI values with lemon addition was observed in rosehip tea ( $7\,707 \times 10^{-4}$ ), fennel tea ( $7\,287 \times 10^{-4}$ ) and sage tea

Table 6. HI values of 2 cups of selected Turkish herbal and fruit tea infusions with and without lemon addition

Tea types	Method	Hazard Quotient ( $10^{-4}$ )				HI ( $10^{-4}$ )
		Ba	Cd	Ni	Pb	
Fennel	I	–	–	9	1 356	1 365
	L	12	3 204	1 168	4 267	8 652
Mint	I	0.5	–	–	–	0.5
	L	0.5	–	2.5	107	110
Sage	I	178	–	430	343	950
	L	184	3 735	297	3 140	7 355
Apple	I	19	–	6	2 726	2 750
	L	722	3 408	499	3 982	8 611
Lemon	I	637	–	–	3 568	4 204
	L	1 418	1 204	294	6 964	9 880
Rosehip	I	30	–	8	3 083	3 120
	L	1 311	3 510	611	5 395	10 827

I – infusions; L – lemon added infusions

( $6\,405 \times 10^{-4}$ ). After the lemon addition, the Hazard Quotient values of Cd and Pb increased more than those of the other risk elements in these teas (Table 6).

## CONCLUSIONS

The lemon effect on risk elements (Al, As, Ba, Cd, Ni, Pb, and Sb) showed differences with the various tea types. After the lemon addition to the teas, the risk elements contents in fennel, apple, and lemon teas revealed similar changes with the increasing levels of Al, Ba, Cd, Ni, and Pb. The As and Sb contents were below the detection limits for these teas with and without lemon. The results of these experiments and HI values (HI value of rosehip tea for 184 ml/day was  $9\,961 \times 10^{-4}$ ) showed that the consumption of more than 184 ml of rosehip tea with lemon per day exceeded the acceptable HI value (1.0). According to the HI values for 200 ml/day, rosehip tea ( $10\,827 \times 10^{-4}$ ) with lemon may present a risk for human health. If the HI values and the increase of HI values of these teas for 200 ml/day after the lemon addition are examined, the consumption of herbal teas and fruit teas with lemon according to the possibility of risk for human body will be ordered from high to low as follows: fennel, sage, and mint for herbal teas; and rosehip, lemon, and apple for fruit teas. In addition to the HI values, the excess amount of daily intake percentage of Ba in lemon and rosehip tea with lemon addition might be caused by the contamination levels in the respective environments.

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