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Full Length Research Paper

Determination of the inorganic constituents of commercial teas and their infusions by the technique of energy dispersive X-ray fluorescence spectrometry

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With the global increase in use of herbal products, such as herbal teas, it has become necessary to obtain information about their inorganic constituents. In this context, the objective of this research was to determine the inorganic constituents of commercial teas (dry leaves) and their infusions in hot water by Energy Dispersive Fluorescence X-ray Spectrometry. In this study, fourteen different varieties of tea were selected from the most commonly encountered and consumed in Brazil. Energy Dispersive Fluorescence X-ray Spectrometer was able to identify in dry tea the elements potassium, sulfur, calcium, copper, phosphorus, iron, and manganese. In the infusions, only sulfur, calcium, and potassium were detected in low concentrations. Potassium has presented the highest concentration in the samples of commercial dry teas and while the infusions, sulfur has presented the highest migration and consequently, it was the most abundant element. Moreover, heavy metals were not found in any concentrations that would be considered harmful to human health.

Key words: Energy dispersive X-ray fluorescence spectrometry (EDXRP), herbal medicine, methods of analysis, mineral elements.

INTRODUCTION

Tea is the second most consumed beverage in the world, behind water (Weisburger, 1997). The use of herbal medicinal teas for the treatment of various human diseases (Eisenberg et al., 1998) increases the demand of timely information about the constituents present in this type of beverage. Takahashi et al. (1992) points out that studies evaluating the incorporation of medicinal plants which serve as raw material for commercial teas presently have adverse effects on human health, as a result of the organic elements or metals present in the tea.

According to Nogueira et al. (1998, 1998, 2002), some minerals play a specific role in the formation of the structure of a plant, however, some species can accumulate excessive amounts of metals in their tissues. Han et al. (2005) confirmed in several herbal teas, the presence of heavy metals like lead and cadmium, which are usually attributed by contamination of soil by industrial waste used as fertilizers, or by polluted water used for irrigation. In this scenario, one way of verifying the constitution of mineral elements in tea is to analyze the infusion. According to Fung et al. (1999), these results should be similar to what is actually consumed by humans, provided by the infusions, where 25 to 84% of the constituents in the leaves are released into the liquid.

With the view to employ new methods of analysis for the identification and measurement of mineral elements present in commercial teas, there is the great potential use of Energy Dispersive X-ray Fluorescence Spectrometry (EDXRF). The conventional EDXRF has only two basic units, the source of excitation and the spectrometric detection system (Figure 1). Normally, a high-resolution semiconductor detector is used [Si (Li)],

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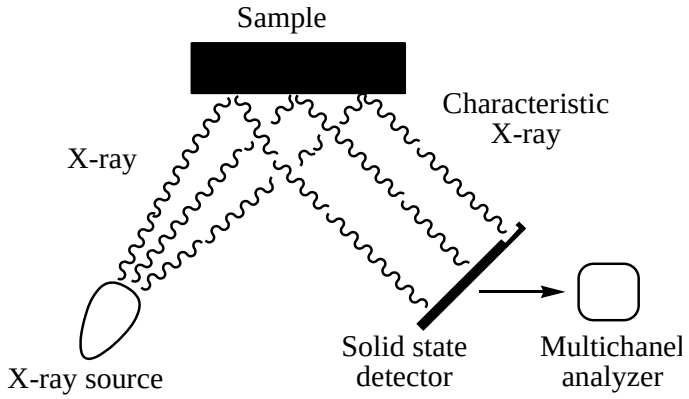

Figure 1. Basic schematic for the EDXRF.

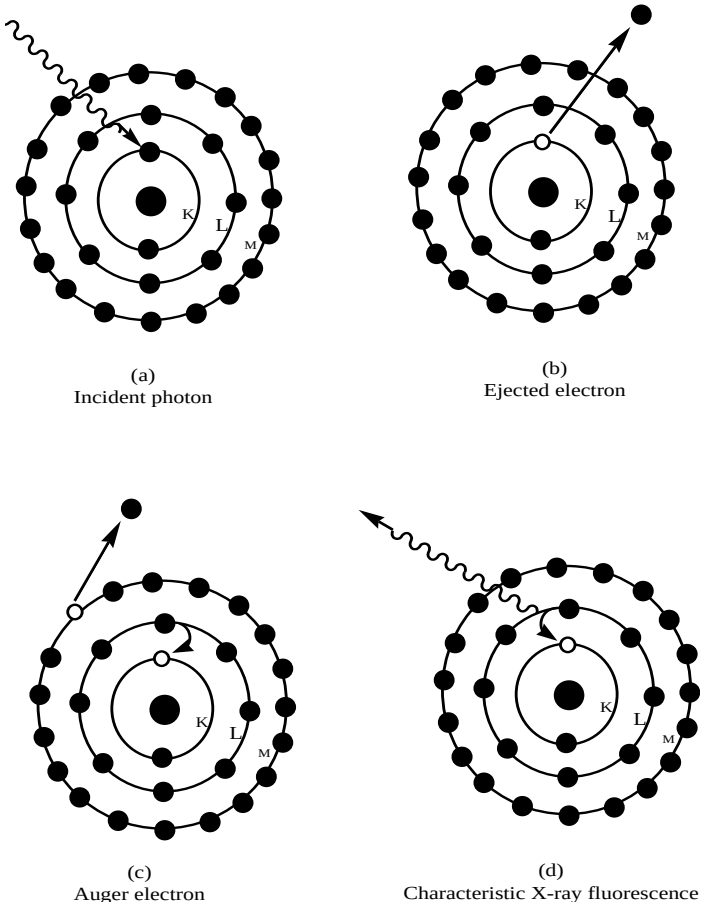

Figure 2. Representation of the fundamentals of X-ray fluorescence.

since the resolution of energy dispersion system is directly related to the intrinsic resolution of the detector. The operating principle of this technique is based on the atomic ionization process followed by the emission of characteristic X-ray (Figure 2) in (a) the atom is subjected to an irradiation process induced by a particle or by an X-ray source, then in (b) a photon with energy higher than the binding energy strikes and, subsequently, ejects an electron from an inner electronic shell. Due this, in (c) a portion of this energy can be absorbed by the rearrangement of electrons (photoelectric absorption), with emission of Auger electros and consequently ionization of the atom and/or in (d) an electron from the outer electron layer drops down to fill the vacancy left by

the ejected electron, releasing an energy equal to the energy difference between these two levels. This process is called characteristic x-ray emission (x-ray fluorescence). As this process involves energy levels that are characteristic for each element, the radiation emitted for each transition is also characteristic. In this way, the energy of radiation can be directly used for the identification of the element in question. Moreover, as the intensity of radiation emitted is a function of concentration, the technique also provides quantitative information regarding to the element (Shoog et al., 2002).

Thus, the technique allows the simultaneous or sequential determination of concentration of all elements between sodium (Na, Z = 11) and uranium (U, Z = 92) without the need to destroy the sample, that is, solely by instrumentally means, without any chemical pretreatment, being able to detect concentrations in the order of 1 to 20 ppm (Boman et al., 1996; Nagata et al., 2001; Wastowski et al., 2010). In this context, the objective of this study was to determine the inorganic constituents of commercial teas and their infusions by EDXRF.

MATERIALS AND METHODS

The study was conducted at the Chemical Research and Analysis Laboratory - LAPAQ at the Federal University of Santa Maria (CESNORS/UFSM) Campus Frederico Westphalen - RS, Brazil. Samples of commercial teas were taken from four different Brazilian brands, totaling 14 samples. For sample preparation, firstly the contents of the bags (4 g) were placed in a forced-air oven set to a temperature of 65°C for 24 h to remove moisture. In accordance with the methodology described by Reto et al. (2008), the infusion samples were obtained by immersing the bags of dry tea in boiling water at 100°C for 10 min.

All the samples were analyzed using an EDXRF, model Shimadzu EDX-720. The experimental settings were: tube voltage of 15 keV (Na to Sc) and 50 keV (Ti to U) with a tube current of 184 and 25 µA, respectively, collimator of 10 mm, real time integration of 300 s, dead time of the detector of 40 and 39% of both tube voltage, under vacuum and Si (Li) detector cooled with liquid nitrogen. The method of the fundamental parameter (FP) (Bona et al., 2007) was used for the analysis. This method allows the determination of a calibration curve for the equipment for each element of interest when a sample of known chemical composition is subjected to well-defined instrument parameters. The calibration curve of the equipment is related to the theoretical calculation of fluorescence intensity and is measured for each element (Bona et al., 2007). In general, the quantitative analysis by EDXRF is performed using a calibration curve with various standards. However, for some applications, it is difficult to achieve sufficient certified standards with matrices similar to the samples, and therefore it is difficult to obtain a good distribution of the data points on the scale for each element to be identified (Omote et al., 1995; Margui et al., 2005, 2009). Han et al. (2006) showed that using the results of the experimental analysis of a variety of different samples, it was possible to obtain results with high precision from FP method, even when only pure element samples are used for calibration. It also clearly illustrates that the FP method can effectively correct the matrix effect. Therefore, for routine mass analysis, if the calibration samples are not available, the FP method can provide relatively precise and quantitative results (Han et al., 2006; Wastowski et al., 2010).

The construction of the calibration curve of an element is usually determined by measuring the characteristic X-ray emission obtained from standards with a known amount of the element of interest. The use of standards prepared in the laboratory from pure elements or compounds was an effective alternative for determining element calibration in XRF systems because they are inexpensive and can be easily prepared (Lopes, 1989). We used the A-750® (part of the equipment) standard to correct the effects of absorption and to calibrate the spectral lines of the analyzed elements. The calibration standards used was an alloy composed of aluminum, tin, magnesium, iron, and copper supplied by the EDX-720 system. Thus, it is possible to determine the composition of the material analyzed.

Approximately, 3 g of commercial dry tea were used for analysis. These samples were put inside a polyethylene cell with 32 mm outer diameter and 23 mm in height with 6 µm thick Mylar® film in its bottom. All elements laying between sodium (Na) to uranium (U) were analyzed, but only the levels of the seven elements with the highest concentrations were quantified and reported, namely: potassium (K) , calcium (Ca), sulfur (S), phosphorus (P), iron (Fe), manganese (Mn), and copper (Cu).

The experimental design utilized complete randomized design (CRD) with four repetitions (dry teas) or three repetitions (infusions). The dry teas which were analyzed were *Baccharis trimera*, *Cymbopogon citrates* Mark 1, *Malus domestica* Borkh, *Citrus spp.*, *Camellia sinensis* with *Caryophullus aromaticus* L, *Melissa officinalis*, *Citrus sinensis* (L.) Osbeck, *C. sinensis* Mark 1, Selvagem® (Tea produced from a mixture of medicinal plants), *Cassia angustifolia*, *Matricaria chamomilla*, *M. domestica* Borkh Unseasoned; *Cymbopogon citratus* 02 Mark 2; and *C. sinensis* Mark 2 (Medicinal plant grown in organic production system).

RESULTS

Samples of dry tea

Table 1 shows the concentrations of S, K, Ca, P, Fe, Mn, and Cu in the 14 samples. The mean concentration for S was 2.29 g/kg, well below the levels found for K and Ca. Among the teas, it was found that the lemon grass tea (*C. citrates* both marks 01 and 02) has the lowest concentrations of S, 1.66 and 1.34 g/kg, respectively. On the other hand, high levels of S were found in Chamomile tea (*M. chamomilla*) (3.57 g/kg) and Apple (*M. domestica* Borkh) (3.32 g/kg), without statistical difference between them. The P which is considered as a macronutrient, since needs to be absorbed by plants in large quantities was found in low concentrations in the dried teas, ranging from 3.45 g/kg in chamomile (*M. chamomilla*) to concentrations lower than 1 mg/kg (not detected) in apple (*M. domestica* Borkh), *M. officinalis*, *C. sinensis* (L.) osbeck, and *C. angustifolia* teas.

The concentrations of the mineral element Fe presented significant variability between repetitions of the tests performed for the teas, elevating the coefficient of variation to 25.5%. The values ranged are from 0.02 g/kg (*M. domestica* Borkh) to 0.75 g/kg (*M. officinalis*), which are significantly higher than those reported by Rozycki et al. (1997) and by Silveira et al. (2009) in their respective studies of *Amaranthus quitensis* (0.064 g/kg) and *Alternanthera tenella* (0.059 g/kg) teas. With respect to

Table 1. Concentrations of sulfur (S), potassium (K), calcium (Ca), phosphorus (P), iron (Fe), manganese (Mn), and copper (Cu) present in samples of commercial teas, analyzed by EDXRF spectrometry.

Tea	Mineral element (g/kg)						
	K	Ca	S	P	Fe	Cu	Mn
Baccharis trimera	15.45	7.55	2.52	1.66	0.18	0.03	0.11
Cymbopogon citratus 01	26.43	4.99	1.66	1.02	0.33	0.03	0.15
Malus domestica Borkh	5.79	0.54	2.30	nd	0.02	0.02	nd
Citrus spp.	13.42	5.94	2.22	1.53	0.35	0.03	0.11
Camellia sinensis with Caryophullus aromaticus L.	18.57	5.13	2.49	1.99	0.19	0.03	1.63
Melissa officinalis	25.74	11.19	2.75	nd	0.75	0.03	0.08
Citrus sinensis (L.) Osbeck	13.21	14.35	2.09	nd	0.18	0.02	0.02
Camellia sinensis	16.67	5.65	2.34	2.18	0.12	0.02	1.21
Selvagem®*	16.21	9.66	2.32	2.20	0.64	0.03	0.90
Cassia angustifolia	11.76	30.84	2.66	nd	0.36	0.02	0.03
Matricaria chamomilla	33.36	9.51	3.57	3.45	0.50	0.03	0.10
Malus domestica Borkh Unseasoned	22.06	5.67	3.33	2.66	0.13	0.04	1.67
Cymbopogon citratus 02	20.58	5.17	1.34	1.37	0.40	0.02	0.21
Camellia sinensis **	18.81	9.37	2.32	2.30	0.43	0.04	2.12
Average	18.43	8.98	2.29	1.45	0.33	0.03	0.60
CV (%)	5.16	8.48	5.94	10.98	25.50	6.66	11.41

nd: Not detected, concentrations less than 1 mg/kg. *Tea produced from a mixture of medicinal plants. **Medicinal plant grown in organic production system.

the trace elements of Mn and Cu, no significant differences in their concentrations were found to be compatible to the results obtained by Reto et al. (2008). However, in organic tea (*C. sinensis*), these elements were found in higher concentrations, especially manganese. In this respect, the occurrence of these elevated concentrations is probably due to fertilizations of crops with organic waste from pig farming, which is known to contain high concentrations of these elements. However, the average concentration of Cu and Mn found in the teas, which are considered heavy metals when found in high concentrations, could not be considered as a threat to human health.

Tea infusions

Considering the results obtained from the tea infusions (Table 2), it was felt that, these depict the real importance of this methodology because they represent the exact constituents of the consumed beverage, the tea. However, the elements such as P, Fe, Mn, and Cu that are present in the dry tea samples were not identified at this stage, probably because they are usually present in very small concentrations (< 1 mg/kg), which are not detectable by the equipment. Fernandes et al. (unpublished data) confirms that concentrations for infusions of the plant tea (*C. citratus*) are very low, only 0.0084 and 0.0016 g/kg for Mn and Fe, respectively.

DISCUSSION

Samples of dry teas

The element K has demonstrated the highest average concentration of 18.43 g/kg, among all the samples of teas. According to Mengel and Kirkby (1987), an important feature of potassium is the high rate at which it is absorbed by plant tissue. Thus, these results can be explained due to the abundance of the element in the tissues of plant species (Martinez et al., 2007), also confirmed in other numerous studies of extraction of metals in vegetables that K is the element with the highest concentration. Nieto (1991) and Nas et al. (1993) respectively reported concentrations of 22 and 22.65 g of K per kg in plants used for food. Silveira et al. (2009) analyzed *A. tenella*, a medicinal plant used for making tea, and found the average concentrations of K to be 45.4 g/kg. With regard to the concentrations of K found in each sample of dry teas, it was found that chamomile tea (*M. chamomilla*) had the highest concentration of 33.36 g/kg and statistically different from the others. On the other hand, tea processed from apple (tea of *M. domestica* Borkh) had the lowest a concentration of K, of 5.79 g/kg.

Similar to K, the element Ca was detected in high concentrations in the samples of teas with an average concentration of 12.61 g/kg. However, among the different teas, the detection and concentrations of calcium varied greatly, concurring with the finding of Silveira et al. (2009). The concentrations of calcium

Table 2. The concentrations of sulfur (S), calcium (Ca), potassium (K), and copper (Cu) in infusions of teas, analyzed by EDXRF Spectrometry.

Infusion	Mineral element (g/kg)		
	S	Ca	K
Baccharis trimera	nd	0.42	0.07
Cymbopogon citratus 01	nd	0.06	0.04
Malus domestica Borkh	1.09	0.06	0.03
Citrus spp.	0.95	0.25	0.31
Camellia sinensis with Caryophullus aromaticus L.	nd	0.61	0.27
Melissa officinalis	1.12	0.47	0.32
Citrus sinensis (L.) Osbeck	1.16	0.59	0.15
Camellia sinensis	1.22	0.70	0.42
Selvagem®*	1.21	0.50	0.12
Cassia angustifolia	1.36	0.33	nd
Matricaria chamomilla	1.22	0.31	0.13
Malus domestica Borkh Unseasoned	1.24	0.76	0.16
Cymbopogon citratus 02	nd	0.07	0.11
Camellia sinensis **	0.81	0.08	0.11
Média Geral	0.81	0.37	0.16
CV (%)	6.75	4.27	7.89

nd: Not detected, concentrations less than 1 mg/kg. *Tea produced from a mixture of medicinal plants. **Medicinal plant grown in organic production system.

ranged from 0.54 g/kg in apple tea (tea of *M. domestica* Borkh) to 30.84 g/kg in *C. angustifolia* tea. With the aim of obtaining an explanation for this variability in the concentration of a mineral within various parts of a species, or between different plant species, Selema and Farago (1996) preformed a literature search and reported that, there is a definite pattern in the absorption of Ca. However, knowing that the concentration of minerals in plants is dependent on various conditions, it can be shown that the concentrations of minerals often assumed a gradient within the same species (Silveira et al., 2009).

Tea infusions

On average, sulfur was the element with the highest concentration in the infusions of teas, with an average concentration of 0.81 g/kg, followed by calcium and potassium with average concentrations of 0.37 and 0.16 g/kg, respectively. Among the infusions, *C. sinensis* tea presented the highest concentration of potassium with 0.42 g/kg followed by the *M. officinalis* and *Citrus* mixed (*Citrus spp*) teas with 0.32 and 0.31 g/kg, respectively (Table 2). These results agree with those of Santos unpublished data, who studied the infusions of four commercial teas and also observed that *C. sinensis* tea had the highest concentration of the inorganic constituent. For the infusions, the green apple tea (*M. domestica* Borkh unseasoned) had the highest concentration of calcium (0.76 g/kg), followed by *C. sinensis* tea (0.70 g/kg).

The results of element migration (Figure 3) demonstrated that the sulfur and potassium were respectively the elements with the highest and lowest solubility in hot water, presenting an average infusion of 35.37 and 0.87%, respectively. The element calcium showed intermediate infusion with an average of 2.93%. In a study of medicinal plants, Silveira et al. (2009) found that the most soluble elements were Si, K, Mg, Fe, Ca, and Al. This variance in results, both in concentration and the solubility of elements is confirmed by several authors (Selema and Farago, 1996; Sovetkina et al., 2001; Collins et al., 2005; Silveira et al., 2009), who found significant variability of values, both in different parts of the plant as well as among the different plants species studied (Figure 3).

From the results, it was concluded that the determination and the knowledge of inorganic constitution of herbal products, such as teas, is extremely important for the standardization of the minerals constituents in commercial products. EDXRF allowed the detection and quantification of the elements of potassium, sulfur, calcium, copper, phosphorus, iron, and manganese in the commercial teas analyzed. As for the infusions, only low concentrations of elements of sulfur, calcium, and potassium were detected. Potassium had the highest concentration in the samples of commercial teas while sulfur was the element that demonstrated the greatest migration from the samples of teas to their infusions. The teas that were evaluated showed no concentrations of

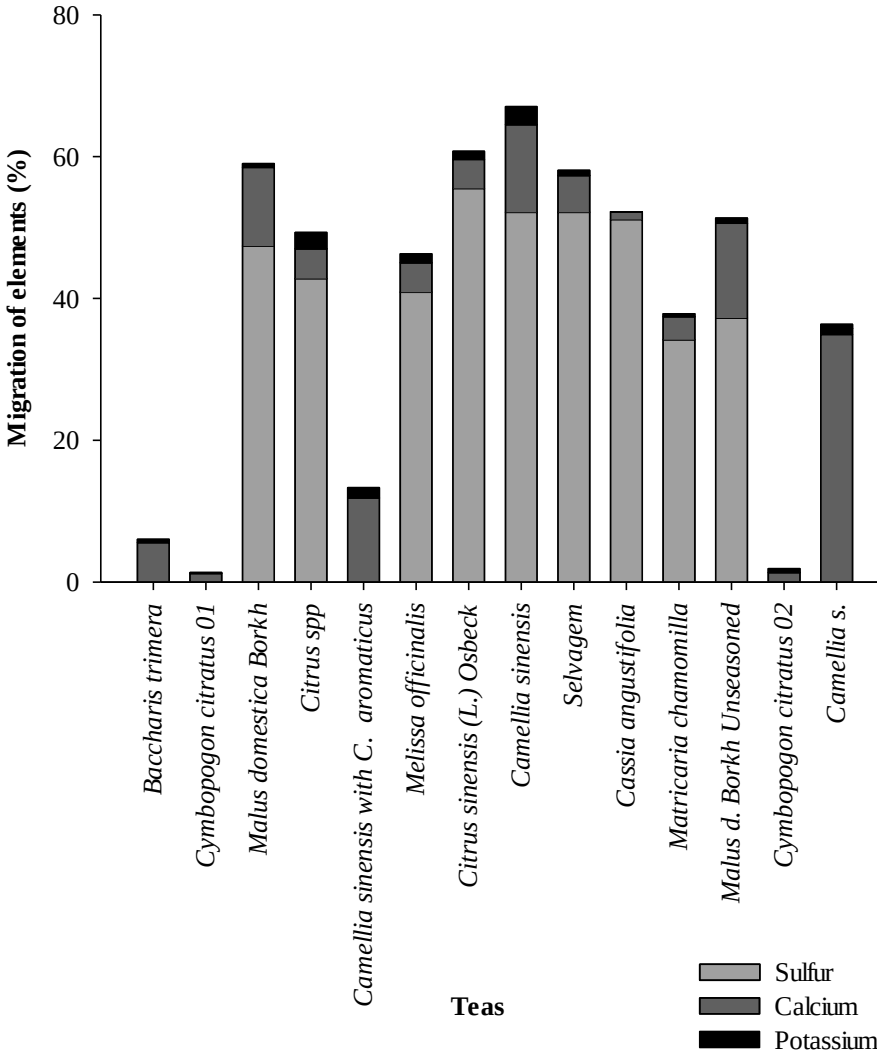

Figure 3. Relative values of the migration of elements of sulfur (S), calcium (Ca), and potassium (K) in infusions of commercial teas, analyzed EDXRF spectrometry.

heavy metals considered harmful to human health.

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