DETERMINATION OF Cl, K, Mg, Mn, Na AND V IN BRAZILIAN RED WINE BY NEUTRON ACTIVATION ANALYSIS

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ABSTRACT

Several studies have been carried out for determining essential elements in foodstuffs due to important nutritional role in human body functions. Such information is crucial to any intervention in the diet of a population, thus becoming representative in the public health field. Trace elements are good indicators of wine's origin and their concentrations can be used as criteria to ensure authenticity, quality and show that the tolerance limits established by law were respected throughout the production process. Although Brazilian wine industry is among the 15 largest in the world, analytical studies on the content of organic and inorganic compounds in wine are still small compared to other major producers. This study aimed to evaluate the elemental concentration of Cl, K, Mg, Mn, Na and V in seven national red wine samples acquired in the markets of Sao Paulo city. The analytical method applied was INAA. Concentrations of the elements in wine samples showed high variation among samples.

1. INTRODUCTION

Wine contains over 600 different substances known and its moderate consumption has health benefits in the prevention of numerous diseases and longer life expectancy, related in particular to the intake of antioxidants such as polyphenolic compounds. Studies have shown that its inorganic composition depends on various factors which are specific for each production area such as grape variety, the potential of the grapes to absorb soil substances, climatic conditions, vineyard soil, vinification practices and storage conditions.

The wine inorganic composition can originate from external factors, such as environmental pollution and absorption of the elements in vineyard soil during grape growth (geonenic); or from the application of fertilizers, pesticides and fungicides to protect the plants. Equipment used in winemaking including storage, handling and bottling processes are also sources of elements or substances due to long contact of wine with the machinery, casks, barrels and pipes [1]. Element content of wines may decrease during clarification and storage processes and the magnitude depends on harvest, grape quality and alcohol content [2].

The inorganic components in wine are important due: organoleptic features that consequently affect the sensor quality, including flavor, freshness, aroma, color and taste [1]; alcoholic fermentation efficiency, regulating the cellular metabolism of yeasts that maintain adequate pH and ionic balance; wine conservation [2]; stability of wine and contribution for the essential elements required of the human body; and may be toxic elements [3]. The analytical

determination of elements present in wine is made to ensure that the tolerance limits established by law are respected throughout the production process [4].

The trace elements are also good indicators of origin of the wine due to their direct relationship with soil composition [5] and their concentrations can be used as criteria to ensure authenticity [2]. World production of grapes exceeds 68 million tons every year [6] and economically, a high quality production brings large incomes. For this reason there is an increasing interest in certificating their geographical origin [7] guaranteed by strict guidelines issued by the competent authorities [8].

The Vitis genus, which has agronomic importance within the family of Vitaceae grapes, are present almost exclusively in the northern hemisphere [9] and consists of about 70 species capable of being hybridized with the Eurasian species *Vitis vinifera*, resulting in many hybrids better adapted to local environments beyond their natural habitat [6]. The species *Vitis Vinifera* is suggested to have first appeared 65 million years ago [9] and is widely used in the fine wine industry worldwide [10]. In Brazil, non-vinifera grapes are refered as *table wine* or *common* wine [11].

Analytical techniques such as atomic absorption spectrometry (AAS), atomic emission with inductively coupled plasma optical emission spectrometry (ICP OES) [15] and more recently the inductively coupled plasm mass spectrometry (ICP-MS) [7], have been applied to quantify chemical components in wines of different origins.

There are virtually no studies that apply Instrumental Neutron Activation Analyses (INAA) specifically in wines. May et al. in 1982 applied INAA to determine elements in French wines demonstrating the technical feasibility [16]. In 1994, at the Laboratorio di Igiene Industriale Italy, Minoia et al. applied the NAA technique to evaluate the importance of determining trace elements in various beverages such as red wine, consequently present in body fluids of consumers [17]. Among its advantages, the analysis does not require large amounts or chemical treatment of the samples and the ability to determine various elements with high sensitivity.

In this paper the concentrations of Cl, K, Mg, Mn, Na and V in seven freeze-dried Brazilian red wine samples from Rio Grande do Sul were determined by Instrumental Neutron Activation Analysis (INAA).

2. MATERIALS AND METHODS

2.1. Sampling

A total of seven Brazilian red wine samples was acquired in the markets of Sao Paulo city. All of them are from Serra Gaúcha, Rio Grande do Sul, in the southern region of Brazil, which is responsible for 90% of the Brazilian wine production (Figure 1). Table 1 shows the description, classification according to grape, sweetness and alcoholic graduation. The fine wine samples presented higher alcoholic level than table samples.

Table 1: Description, classification and origin of the analyzed wines

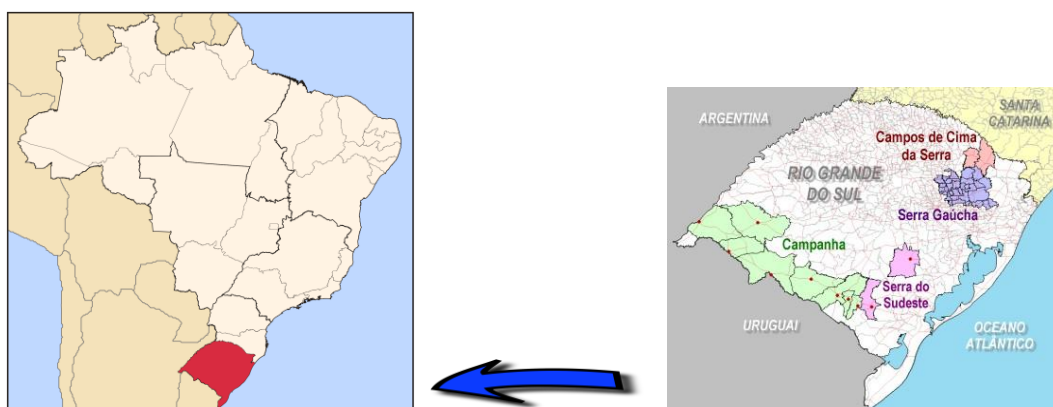| Wine	Grape	Sweetness	Alcoholic Graduation (% Vol.)	Origin
A	Table	Dry	10.5	Flores da Cunha - RS
B	Table	Dry	10.5	Bento Gonçalves- RS
C	Table	Dry	10.5	Flores da Cunha - RS
D	Fine	Dry	13.0	Bento Gonçalves- RS
E	Table	Sweet	10.7	Flores da Cunha - RS
F	Table	Dry	10.8	Flores da Cunha - RS
G	Fine	Sweet	12.5	Bento Gonçalves- RS

Figure 1: Serra Gaúcha (RS), in southern region of Brasil

2.1.1. Sample preparation

Firstly, the wine samples were transferred from glass bottle to polyethylene recipients. These recipients with the wine samples were weighed and stored in freezer for 24 hours. Before freeze-drying these recipients were placed in a liquid nitrogen bath to keep samples in solid state. After that, these samples were pre-concentrated through freeze-drying process for 8 hours at -50 $^\circ$C and 49 μbar in the ModulyoD Freeze Dryer (Thermo Electron Corporation). The recipients were then weighed again at the room temperature. After freeze-drying, three aliquots of 200 μl of each sample were pipetted on sheets of previously weighed 41 Whatman filter paper. Filter papers loaded with the samples were dried under infrared lamp for 1 hour, weighed and packed in demineralized polyethylene bags. Density of wines was measured on previously calibrated volumetric flasks.

2.2. Preparation of Elemental Synthetic Standards

Element synthetic standards were prepared from certified standard solutions of Spex CertiPrep (USA) previously diluted to appropriate concentrations and used to quantify the element concentrations by INAA. About 25 to $100 \mu l$ of these standard solutions were pipetted on sheets of 41 Whatman paper. Filter papers loaded with the standards were dried at room temperature or under infrared lamp and packed in demineralized polyethylene bags.

2.3. Certified Reference Material

Aliquots of 100 mg of the Mixed Polish Herb (INCT-MPH-2) reference material provided by the Nuclear Energy Institute of Poland was weighed and packed in previously demineralized polyethylene bags to verify the accuracy of the procedure.

2.4. Blank Preparation

Five sheets of previously weighed 41 Whatman filter paper were packed in demineralized polyethylene bags at the same size of CRM and samples to ensure the same counting geometry. Blanks were analyzed to quantify the possible contaminants of filter paper and polyethylene bags.

2.5. Irradiation and Counting

Samples, CRM and synthetic elemental standards were packed in previously demineralized polyethylene bags and transferred into the irradiation sites of IEA - R1 nuclear research reactor operating at a power of 4.5 MW by means of a pneumatic transfer system. The irradiation time was 20 seconds under a thermal neutron flux of 1.0×10^{12} n cm^{-2} s^{-1}.

After irradiation, activities were measured twice, under the same counting geometry: first for 5 minutes and then after 30 min to allow decay of interfering short-lived radionuclides. The induced gamma ray activities were measured by HPGe detector model GC 19020 from Canberra, coupled to a digital spectrum analyzer DSA 100. The system resolution used was 1.00 keV for the 121.97 keV peak of ^{57}Co and 1.80 keV for the 1332.49 keV peak of ^{60}Co. Genie 2000 version 3.1 software from Canberra were used for data acquisition and spectrum processing.

The ^{38}Cl, ^{42}K, ^{27}Mg, ^{56}Mn, ^{24}Na, and ^{51}V radionuclides were identified in the spectrum by their half-life and the gamma ray energy. The concentration of the elements in the samples was obtained by comparing the area of their peaks with the peaks of the elemental standard solution that are irradiated together, by applying equation 1 that follows:

$$C_s = \frac{m_e \, A_s \, e^{0,693 \, (tds-tde)/t_{1/2}}}{M_s \, A_e} \tag{1}$$

Where s and e refers to sample and standard, respectively:
M_s = sample total mass; m_e = element mass in the standard; C_s = element concentration in the sample; $t_{1/2}$ = half-life of the radioisotope; td = decay time.

3. RESULTS AND DISCUSSION

3.1. Certified Reference Material (CRM) Analysis

In order to verify precision and trueness of the applied methodology, the Mixed Polish Herb CRM was analyzed. Results obtained in the CRM analysis are shown in Table 2. Relative standard deviation (RSD) of the measurements was less or equal than 10%, except for V, which can be considered to have a good precision for this level of the elements in the sample. The higher RSD result for V measurements can be explained by the low concentration of the element in the CRM.

The relative error (ER %) values of the elements K, Mg, Mn and Na were less than 5% and it can be considered to be in good agreement to certified values. Measurement of Cl and V demonstrated 11% of ER, but it is acceptable to consider them to have a good accuracy too.

Table 2: Concentrations of the elements in Mixed Polish Herb CRM

Element	$(\bar{X} \pm SD)$ [a]	RSD [c] (%)	RE [d] (%)	Certified Values [e] (mg kg^{-1})
Cl	2532 ± 204	8	-11	2840 ± 200
K	18531 ± 1451	8	-3	19100 ± 1200
Mg	2819 ± 292	10	-3	2920 ± 180
Mn	182 ± 18	10	-5	191 ± 12
Na	363 ± 28	8	4	(350) [f]
V	0.84 ± 0.15	17	-11	0.95 ± 0.16

a. Arithmetic mean and standard deviation for n=10. Results in mg kg^{-1}; c. Relative standard deviation; d. Relative error; e. uncertainty expanded (k=2); f. Informative value

3.1. Wine samples Analysis

Data obtained in the analysis of the freeze-dried wine samples are presented in Table 3. In order to calculate the concentration of the elements in red wine before pre concentration, it was considered density and loss of mass in the freeze-drying processes, which varied between of $0.995 - 1.035$g/ml and $31 - 60\%$, respectively. Results obtained are shown in Table 4.

The results obtained for blank analysis are shown in the Table 5. These results were considered non-significant due to the too low values when compared with the concentrations of the K, Mg, Mn and V elements in the wine samples. For Cl and Na the blank values were considered to calculate the concentrations in wine samples.

Concentrations of the elements in wine samples showed high variation among samples. Sample B presented the highest concentration values for Cl, K and Na, whereas sample E presented the lowest values for all the elements.

Table 3: Concentrations of the elements in freeze-dried red wine samples

Element	Concentrations in mg kg^{-1}.					
	Mg	Cl	V	Mn	Na	K
A	4355 ± 322	1486 ± 119	5.4 ± 0.4	83 ± 8	791 ± 9	37693 ± 1751
B	4041 ± 440	1746 ± 299	1.7 ± 0.6	95 ± 20	905 ± 210	46828 ± 7979
C	3809 ± 432	875 ± 153	2.0 ± 0.3	45 ± 8	419 ± 210	23507 ± 9956
D	3066 ± 404	919 ± 94	0.388 ± 0.009	106 ± 3	829 ± 45	39960 ± 2131
E	740 ± 41	204 ± 41	0.332 ± 0.003	14 ± 1	65 ± 10	6381 ± 383
F	4134 ± 160	1607 ± 185	3.02 ± 0.10	76 ± 7	727 ± 69	34866 ± 3314
G	1174 ± 79	2487 ± 94	0.6 ± 0.1	27 ± 3	390 ± 27	11213 ± 1030

Table 4: Concentrations of the elements in red wine samples

Element	Concentrations in mg L^{-1}					
	Mg	Cl	V	Mn	Na	K
A	2940 ± 218	1003 ± 80	3.6 ± 0.3	56 ± 5	534 ± 6	25446 ± 1182
B	2775 ± 302	1199 ± 205	1.2 ± 0.4	66 ± 14	621 ± 144	32149 ± 5478
C	2413 ± 274	554 ± 97	1.3 ± 0.2	28 ± 5	265 ± 133	14896 ± 6309
D	1936 ± 255	580 ± 59	0.24 ± 0.01	67 ± 2	523 ± 28	25238 ± 1346
E	370 ± 185	102 ± 78	0.166 ± 0.002	6.9 ± 0.6	33 ± 5	3187 ± 191
F	1896 ± 74	737 ± 85	1.39 ± 0.04	35 ± 3	334 ± 32	15994 ± 1520
G	475 ± 32	1005 ± 38	0.25 ± 0.02	11 ± 1	158 ± 11	4533 ± 416

Table 5: Concentrations of the elements in blank

Element	Results in mg kg^{-1}.	
	$\bar{X}$[a]	SD[b]
Cl	108	9.8
Mn	0.06	0.01
Na	40	1

a. Arithmetic mean (n=5 samples); b. Standard deviation

Quantities of Mn are one of the minor element present, usually influenced by the composition of the vineyard soil or rootstocks of the vine because it modifies the element uptake capacity of the grapes [8] and its responsible for a phenomenon called "browning" - which consists in the oxidation of the wine compounds. Results of Mn concentrations obtained were one of the lowest indeed, ranging from 6.9 to 67 mg L^{-1}, but it is still higher when compared to literature where concentrations are in the range of 0.1–10 mg L^{-1}[2]. This oxidation leads to changes in stability of wines, loss of freshness and precipitation of condensed tannins.

K, Mg and Na are the major elements present in wines and regulate cytoplasmic pH and the ionic balance of the yeasts, ensuring better alcoholic fermentation efficiency [18]. Their concentrations in the literature are in the range of $10–10^3$ mg L^{-1} [2]. Especially for Na, treatments with sodium bisulfites or insufficiently purified bentonites may increase its concentrations to $10 – 40$ mg L^{-1} [19].

Concentrations of K in the literature are between 10 and 1000 mg L^{-1}[2]. K should be the predominant cation in fine wines such as the samples D and G, but it can be also derived from metabisulfites and carbonates, added in the vinification processes [19]. The concentrations of K and Mg may also decrease in order of its consume by yeasts in fermented musts and wines [2]. These factors may explain the samples A and B to have the highest values for K concentrations (25446 mg L^{-1} and 32149 mg L^{-1}, respectively) and E and G to have shown the lowest values.

Concentration of Mg in sample A was 2940 mg L^{-1} whereas in sample E it was 370 mg L^{-1}. Mg concentrations in wines do not decrease during fermentation and aging, as all magnesium salts are soluble. In literature it is at levels in the range of $10 – 1000$ mg L^{-1} [2]. Mg concentration comes from the grape must, but may be increased, in order of the addition of desacidificants [8].

Na is a natural constituent of soil or can be derived from illicit chloride addition in order to salting or clarifying treatments and its concentration is constant during wine production since it has less influence on yeast growth and performance [2]. Results of Na concentrations obtained were at the range of $33 – 621$ mg L^{-1} and it is according to those found in the literature, which are in the range of $10 - 10^3$ mg L^{-1}.

Concentrations of V obtained in the results are not in agreement with those found in the literature ($0.1– 0.40$ mg L^{-1}) [2]. Sample A presented the highest value for V concentration (3.6 mg L^{-1}). Content of V presents in wines comes, more probably, from interaction of musts and wines with metallic containers than from soil of origin [8] and this may explain the high variation.

Inorganic anions such as Cl^- in wine correspond to the presence of slightly soluble salts such as lead, silver and mercury. Chloride concentrations in wine are usually below 50 mg L^{-1}(expressed in sodium chloride) and may increase to 1000 mg L^{-1} when vineyard is grown closed to the sea [19]. Sample B presented the highest value for Cl, whereas sample E showed the lowest value (1199 ± 205 mg L^{-1} and 102 ± 78 mg L^{-1}, respectively).

CONCLUSIONS

The wide variation of the concentrations among the samples show that the inorganic composition depends on various endogenic and exogenic factors, such as soil of origin, grapes capacity to absorb these elements, use of substances to treat or protect the plants, environmental pollution, climatic conditions, storage conditions and contact with equipments in winemaking process. Further analyses on the soils of origin and vinification processes would be necessary for better understanding about inorganic composition of the samples.

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