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Analytical Methods

Flavoured *versus* natural waters: Macromineral (Ca, Mg, K, Na) and micromineral (Fe, Cu, Zn) contents

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ABSTRACT

Macro (Ca, Mg, K, Na) and micromineral (Fe, Zn, Cu) composition of 39 waters was analysed. Determinations were made by atomic flame spectrophotometry for macrominerals and electrothermic atomisation in graphite furnace for microminerals.

Mineral contents of still or sparkling natural waters (without flavours) changed from brand to brand. Mann–Whitney test was used to search for significant differences between flavoured and natural waters. For that, the concentration of each mineral was compared to the presence of flavours, preservatives, acidifying agents, fruit juice and/or sweeteners, according to the labelled composition.

The statistical study demonstrated that flavoured waters generally have increased contents of K, Na, Fe and Cu. The added preservatives also led to significant differences in the mineral composition. Acidifying agents and fruit juice can also be correlated to the increase of Mg, K, Na, Fe and Cu. Sweeteners do not provide any significant difference in Ca, Mg, Fe and Zn contents.

1. Introduction

Water makes up more than two thirds of the human body, and it is the most consumed drink in the world. To answer to consumer's preferences, industries have applied several technical improvements to plain water. Today, a significant part of marketed water is flavoured. It consists in the addition of flavours, juices, bioactive compounds, preservatives and/or sweeteners that provide singular tastes and smells appreciated by consumers.

In the case of flavoured waters, either mineral or spring sources are used, both having important mineral contents. According to Food and Drug Administration (FDA, 2002), mineral water arises from a geologically and physically protected underground source, characterised by constant levels and relative proportions of minerals and trace elements at the source. Spring water is derived from an underground formation from which water flows naturally to the surface at an identified location.

Minerals are necessary for human life and play important roles in metabolic functions (Biziuk & Kuczynska, 2007) such as, maintenance of pH, osmotic pressure, nerve conductance, muscle contraction, energy production, and in almost all other aspects of life. Depending on the amounts needed, minerals can be divided into macro (g or mg/day) and microminerals (few mg or μg/day). Physiologically, the most important macrominerals are Ca, K, Na and Mg, and the same for Fe, Cu and Zn as microminerals (Silvera & Rohan, 2007).

Bioavailability of minerals is affected by several factors. Host factors can be defined as any attribute that can influence the amount of metal exposure, uptake, absorption, biokinetics and susceptibility of an individual. Such factors include age, gender, size and weight, nutritional status, genetics and some behaviours (Robson, 2003).

Although minerals are essential to normal health and development, they can become toxic in higher amounts. Risk assessments of chemical elements show high intakes that result in toxicity or nutritional problems related to low or no intakes (Goldhaber, 2003). So, it is important to establish an adequate intake of certain substance to avoid adverse health effects (Nasreddine & Parent-Massin, 2002). To answer this goal, the Joint Expert Committee on Food Additives (JECFA) of the Food and Agriculture Organisation of the United Nations and the World Health Organisation (FAO/WHO, 2000) established acceptable or tolerable intakes for substances that exhibit thresholds of toxicity. Provisional Tolerable Daily Intake (PTDI), calculated on a daily basis for certain substances that do not accumulate in the human body, is the reference value that indicates the safe level of intake. US Environmental Protection Agency (EPA) has also carried out the Reference Dose

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Table 1
Recommended dietary allowances (RDA), provisional tolerable intakes (PTDI) and reference dose (RfD) for the studied minerals.

Mineral	RDA (mg/day)	PTDI (mg/$kg_{bodyWeight}$/day)	RfD (mg/kg/day)
Ca	1000[a]	–	–
Mg	420[male;a] 320[female;a]	–	–
K	4700[b]	–	–
Na	1500[b]	–	–
Fe	8[male;a] 18[female;a]	0.8[c]	–
Zn	11[male;a] 8[female;a]	1.0[d]	0.3[f]
Cu	0.9[a]	0.5[e]	–

Adult Values (male or female) with 31–50 years old.
–, Not available.
[a] Institute of Medicine from United States (2001).
[b] Institute of Medicine from United States (2004).
[c] WHO/FAO (1983).
[d] WHO (1982a).
[e] WHO/FAO (1982b).
[f] Environmental Protection Agency of United States, EPA (2005).

(RfD), being the amount of a daily human exposure (including sensitive subgroups) to a certain compound, without an appreciable risk during a lifetime (EPA, 1993). RfD is generally expressed in mg/$kg_{bodyweight}$/day. US Food and Nutrition Board of the Institute of Medicine (FNB/IOM) set forward the Recommended Dietary Allowance (RDA) as the average daily intake that meets the nutrient requirements of nearly all healthy individuals in a particular life stage and gender (Institute of Medicine from United States, 2007). Table 1 presents the different values established for the aforementioned minerals.

Several analytical methods have been developed to determine the mineral contents in biological, food and environmental samples. The most commonly employed techniques are described below. Inductively coupled plasma mass spectrometry (ICP–MS) (Liu, Chen, Yang, Chiang, & Hsu, 2007), inductively coupled plasma atomic emission spectroscopy (ICP–AES) (Mehra & Baker, 2007), these techniques allow a multielement analysis; however the equipment used is very expensive and also have high operation coasts. Atomic absorption spectrophotometry (AAS) with flame or electrothermic atomisation in graphite furnace (Galani-Nikolakaki, Kallithrakas-Kontos, & Katsanos, 2002; Tamasi & Cini, 2004). The AAS based technique is robust, well establish, easy to use, and presents good detection and quantification levels, mg/L and µg/L for flame or graphite furnace technique, respectively. The use of voltammetry (Melucci, Torsi, & Locatelli, 2007) to quantify metals is an inexpensive and fast technique but normally associated with the use of mercury electrode considered as toxic and environmental unfriendly.

The present study aims to evaluate the contents of Ca, Mg, Na, K, Cu, Fe and Zn in 39 mineral and spring water samples, with and without flavours. Atomic absorption spectrophotometry with flame or electrothermic atomisation in graphite furnace was the implemented methodology. A nutritional and statistic study was carried out to compare these water kinds.

There are no known reports, or any type of evaluation, concerning the mineral contents of these flavoured waters. So, the presented research work is crucial to consumer's information about the advantages/disadvantages of the consumption of these beverages.

2. Materials and methods

2.1. Reagents and equipment

The water used had ultrapure quality (18.2 $M\Omega cm^{-1}$) and was obtained from a Millipore Simplicity 185 system.

All reagents and solvents used were suprapure grade and acquired from Merck, except CsCl that was from Sigma. Standard solutions of each element (Ca, Mg, Na, K, Cu, Fe and Zn) were daily prepared by dilution of the corresponding stock solutions (1000 mg/L), with water and 0.1% (v/v) nitric acid, and stored in polyethylene bottles.

$Mg(NO_3)_2$ (0.1%; v/v) was used as matrix modifier for the determination of Fe and Zn and CsCl (0.1%; v/v) to evaluate Na and K contents.

All glassware and polyethylene vessels were soaked with 10% HNO_3, at least overnight, and then rinsed with ultrapure water prior to use.

Macrominerals were quantified in a Perkin Elmer AAnalyst™ 200 spectrophotometer with an air–acetylene flame. Ca, Mg, Na and K were analysed at wavelengths of 422.7, 285.2, 589.6 and 766.5 nm, respectively.

Microminerals (Fe, Zn and Cu) were quantified in an Analytik Jena Zeenit 650 spectrophotometer with electrothermic atomisation in graphite furnace (wavelengths of 248.4, 213.9 and 324.8 nm, respectively) equipped with an Analytik Jena MPE60 autosampler. Pyrolytically coated graphite tubes with integrated pin platform (Analytik Jena AG) were used. Specific interferences from the matrix were not observed in all samples and the Zeeman background correction was sufficient. Hollow cathode lamps were used (Varian). A stream of ultrapure argon at 5.5 bar was used in the electrothermic determinations, except in the Auto-zero and atomisation step.

2.2. Samples and sample preparation

Thirty-nine water samples (flavoured and the natural ones) corresponding to 10 different brands (mineral and spring) were collected in several supermarkets in the North of Portugal. Each brand (still or sparkling) had different flavours and aromas.

Table 2 summarises the labelled information, namely the presence of vitamins, sweeteners and preservatives.

All samples were acidified with suprapure HNO_3 (1 mL/L) and stored in sealed polyethylene bottles maintained at 4 °C. The gas of sparkling water was removed by sonication, before HNO_3 treatment or acidification.

2.3. Validation of the methodology

Calibration standards were daily prepared (all samples were determined in triplicate). The proposed methods were validated by linear range, limit of detection (LOD), limit of quantification (LOQ), precision and accuracy. LOD and LOQ were defined, respectively, as three and 10 times the standard deviation of 10 blank signals divided by the slope of the calibration plot (Miller & Miller, 2000). The precision was investigated considering the intra-day and inter-day determinations of standard solutions and expressed by relative standard deviations (RSD). For intra-day evaluation each concentration was assessed by three measurements, at three times along a working-day. The inter-day precision measurements were performed over a period of one week. Accuracy and reproducibility were checked by the recovery (REC), the relative error (RE) and the RSD.

2.4. Data analysis

All results were expressed as mean ± standard deviation. In the statistical analysis, data were presented as median (1st quartile–3rd quartile). The significance of the differences between natural waters with and without natural gas was tested by Mann–Whitney test. Comparisons between natural water group and the respective flavoured water group were carried out by Wilcoxon test (dependent samples). All statistical analysis was performed using

Table 2
Label information in bottled flavoured waters evaluated.

Brand	Sample	Flavour	Composition (mg/L)	Other ingredients
Still water				
A Mineral	1	Lemon	Energy value 1.3 kcal/100 mL, Na^{+} (<200), proteins (<1000); carbohydrate (1000), lipids (<1000)	Fibres (1%), wheat dextrin (0.1%) citric acid, potassium sorbate, sodium benzoate, sodium citrate, acesulfame-K
	2	Mango		
	3	Strawberry		
	4	Natural	Total dissolved solids (47), SiO_2 (12.7), Ca^{2+} (0.75), F^{-} (<0.08), NO_3^{-} (1.7), Na^{+} (6.9), Mg^{2+} (1.7), Cl^{-} (9.4), HCO_3^{-} (11.6), pH 5.7	
B Spring	5	Pineapple/orange		Apple juice concentrate, calcium lactate, citric acid, potassium sorbate, sodium benzoate, acesulfame-K, aspartame
	6	Lemon		Apple juice concentrate, citric acid, potassium sorbate, sodium benzoate, vitamins: niacin, pantothenic acid, B_6, folic acid, biotin, B_{12}, acesulfame-K, aspartame
	7	Natural	Total dissolved solids (52), SiO_2 (21.5), Na^{+} (<5.5), Ca^{2+} (<4.3), HCO_3^{-} (<24), Cl^{-} (<30); pH 5.8–6.9	
C Mineral	8	Lemon/magnesium	Energy value 13 kcal/100 mL, proteins (<1000), sugar (23000), Sat. fatty acids (<500), fibres (<1000), Na^{+} (12), Mg^{2+} (450)	Fruit juice concentrate, citric acid, potassium sorbate, dimethyl dicarbonate, magnesium carbonate, ginseng, vitamins (mg/100 mL): B_3 (2.7), B_5 (0.9), B_6 (0.3), B_8 (0.022), B_9 (0.03), B_{12} (1.5×10^{-4})
	9	Apple/white tea	Energy value 17 kcal/100 mL, proteins (<1000), sat. fatty acids (<500), sugar (43000), fibres (<1000), Na^{+} (14), Ca^{2+} (1200)	Fruit juice concentrate, calcium lactate, citric acid, malic acid, potassium sorbate, vitamins (mg/100 mL): B_3 (2.7), B_5 (0.9), B_6 (0.3), B_8 (0.022), B_9 (0.03) and B_{12} (1.5×10^{-4})
	10	Pineapple/fibre	Energy value 9 kcal/100 mL, proteins (<1000), sugar (23000), sat. fatty acid (<1000), fibres (9000), Na^{+} (14)	Fruit juice concentrate, wheat dextrin (0.9%), citric acid, potassium sorbate, dimethyl dicarbonate, L-carnitine (200 mg/L)
	11	Natural	Total dissolved solid (45), SiO_2 (18), HCO_3^{-} (5.1), Cl^{-} (7.4), NO_3^{-} (2.1), Ca^{2+} (0.8), Na^{+} (5.8), Mg^{2+} (0.5), pH 5.7	
D Mineral	12	Apple	Energy value 0.9 kcal/100 mL	Citric acid, dimethyl dicarbonate, sodium benzoate, sucralose, acesulfame-K
	13	Orange/peach		
	14	Lemon		
	15	Natural	Conductivity (515 μS/cm), HCO_3^{-} (315), SO_4^{2-} (25), Cl^{-} (10), Ca^{2+} (83), Mg^{2+} (24), Na^{+} (4.7)	
Sparkling water				
E Mineral/added gas	16	Lemon	Energy value 1.4 kcal/100 mL, proteins (<1000), carbohydrates, (<100) lipids (<1000)	Lemon juice, carbon dioxide, citric acid, sodium citrate, potassium sorbate, sodium benzoate, acesulfame-K, aspartame, vitamins (mg/100 mL): B_3 (2.7), B_{12} (0.15)
	17	Orange/raspberry		
	18	Peach/pineapple		
	19	Guava		
	20	Natural	Total dissolved solids (47), SiO_2 (12.7), Ca^{2+} (0.75), F^{-} (<0.08), NO_3^{-} (1.7), Na^{+} (6.9), Mg^{2+} (1.7), Cl^{-} (9.4), HCO_3^{-} (11.6)	
F Mineral/natural gas	21	Lemon/green tea	Energy value 19 kcal/100 mL, proteins (1000), sugar (43000), lipids (<500), Na^{+} (600)	Lemon, apple and pear juice, fibres (<1000), citric acid
	22	Raspberry/ginseng		
	23	Peach/white tea		
	24	Mango/ginkgo biloba		
	25	Melon/mint		
	26	Natural	Total dissolved solids (3011), SiO_2 (62), HCO_3^{-} (2125), Cl^{-} (31), NO_3^{-} (0.3), Ca^{2+} (103), Na^{+} (622), Mg^{2+} (28), pH 6.1	
G Mineral/added gas	27	Lemon	Energy value 0.4 kcal/100 mL	Citric acid, vitamin C (12 mg/250 mL), potassium sorbate, acesulfame-K, sucralose
	28	Lime		
	29	Apple		Citric acid, vitamin C (12 mg/250 mL), potassium sorbate, acesulfame-K, sucralose
	30	Peach		
	31	Natural	Total dissolved solids, 180 °C (497), Cl^{-} (78), SO_4^{2-} (22), HCO_3^{-} (373), Na^{+} (37), Ca^{2+} (105), Mg^{2+} (29), pH 5.43	

(*continued on next page*)

Table 2 (*continued*)

Brand	Sample	Flavour	Composition (mg/L)	Other ingredients
H Mineral/natural gas	32	Lemon	Energy value 4 kcal/100 mL proteins (<1000), carbohydrates (10000), lipids (<1000)	Lemon and apple juice, citric acid, vitamin C (30 mg/100 mL), sodium benzoate, potassium sorbate, aspartame
	33	Natural	Total dissolved solids (2776), SiO_2 (37), HCO_3^- (1954), Cl^- (34), NO_3^- (0.3), Ca^{2+} (75), Na^+ (607), Mg^{2+} (14), pH 6.1	
I Mineral/natural gas	34	Lemon	Energy value 2 kcal/100 mL, carbohydrates (5000)	Lemon and apple juice, citric acid, aspartame, sodium benzoate
	35	Green apple	Energy value 4 kcal/100 mL, proteins (500), carbohydrates (9000)	Apple juice, citric acid, sodium benzoate, sucralose
	36	Strawberry	Energy value 2 kcal/100 mL, carbohydrates (5000)	Apple and strawberry juice, citric acid, sodium benzoate, aspartame
	37	Natural	CO_2 free (2260), total dissolved solids (3535), SiO_2 (26.7), Cl^- (145), HCO_3^- (2393), Na^+ (741), Mg^{2+} (35.2), Ca^{2+} (116)	
J Spring/added gas	38	Lemon	Energy value 4 kcal/100 mL, proteins (<1000), carbohydrates (<1000), lipids (<1000)	Lemon juice, citric acid, sodium citrate, potassium sorbate, sodium benzoate, aspartame, acesulfame-K
	39	Natural	Total dissolved solids (180 °C) (21), SiO_2 (8.5), HCO_3^- (5.8), Cl^- (3.4), Ca^{2+} (1.2), Mg^{2+} (0.3), K^+ (0.2), Na^+ (2.5)	

the Statistica7 software, $p < 0.05$ was considered statistically significant.

3. Results and discussion

Macro (Ca, Mg, K, Na) and microminerals (Fe, Cu, Zn) are essential, in different amounts, to normal human development. So, an adequate intake from dietary sources is very important to avoid deleterious effects on human health and general well-being.

Before the evaluation of the mineral content in the samples it was necessary to implement and validate the employed methodologies.

3.1. Minerals quantification

Table 3 summarises the data from calibration curves and the performance characteristics for the seven minerals in study. Linearity ranges were from 0.10 to 5.00 mg/L in macrominerals and from 1.0 to 20.0 μg/L in microminerals. The calculated LOD values ranged from 4.6 to 30.2 μg/L for macrominerals, and 0.21 to 1.7 μg/L for microminerals. LOQ values range from 15.2 to 100.2 μg/L and 0.7 to 5.5 μg/L for macrominerals and microminerals, respectively.

Precision and accuracy values are shown in Table 3. No significant differences were found between intra-day and inter-day experiments. RSD values ranged from 1.0% to 4.3%, and confirmed the high precision of the method. REC and RE values assessed the accuracy of the results. RE were always <10.0% and recovery trials ranged from 99% to 110%, confirming the accuracy of the implemented method.

3.2. Global discussion

Table 4 shows the minerals contents of still and sparkling waters, respectively. The mineral composition of the natural waters (without flavours) changed from brand to brand. This was due to their different natural origins, from different geological structures.

According to the values described in the label (Table 2) it is possible to discriminate three groups in natural waters, attending to the total dissolved solids values:

- one with reduced values ranging from 21 to 47 mg/L corresponding to natural waters 4, 7, 11, 20 and 39 (50% of the samples considering the flavoured ones). This group included all spring waters studied (samples 7 and 39); sample 39 had added gas. The other samples included in this group were mineral waters and only sample 20 had added gas;
- another group included two samples with intermediate values (samples 15 and 31) with total dissolved solids ranging 500 mg/L. This group corresponds to 25% of the total samples analysed;
- the third group included mineral waters with natural gas and their total dissolved solids ranged from 2776 to 3535 mg/L (samples 26, 33 and 37).

From all the evaluated samples, the excessive consumption of the mineral waters, pertaining to this group, with or without flavours, can contribute to the development of health problems namely kidneys disorders. Considering the values of Table 1, three bottles of this water (about 1 L) per day correspond to an ingestion of a 1/3 of the RDA for Na.

Taking into account the macromineral values presented in the label and the determined ones (Tables 2 and 4), in general, there are in agreement. All label samples described the contents of Ca, Mg and Na and only one (sample 39) presented K contents. None of them expressed micromineral contents.

Table 3
Calibration curves, limit values, precision and accuracy obtained for the minerals studied.

Parameters	Ca	Mg	K	Na	Fe	Zn	Cu
Linear concentration (μg/L)	1000.0–5000.0	100.0–400.0	400.0–1500.0	400.0–1500.0	6.0–20.0	2.50–20.0	1.0–10.0
Slope (Abs μg^{-1} L)	5.2 ± 0.2 ($\times 10^{-5}$)	9.3 ± 0.5 ($\times 10^{-4}$)	1.9 ± 0.8 ($\times 10^{-4}$)	2.7 ± 0.2 ($\times 10^{-4}$)	0.0118±0.0002	11.08 ± 0.04	0.014 ± 0.001
Intercept (Abs)	0.010 ± 0.006	2.4 ± 0.1 ($\times 10^{2}$)	1.9 ± 0.7 ($\times 10^{-3}$)	−0.02 ± 0.02	$1.18 \times 10^{-2} \pm 0.02 \times 10^{-2}$	0.9 ± 0.4	0.003 ± 0.002
Correlation coefficient (*n* = 3)	0.997	0.995	0.997	0.992	0.9994	1.00	0.9981
LOD (μg/L)	30.2	4.6	27.3	17.1	1.7	0.59	0.21
LOQ (μg/L)	100.2	15.2	91.0	57.1	5.5	1.90	0.7
Intra-day studies[a]							
Added (μg/L)	1000.0	300.0	1200.0	1000.0	12.0	20.0	5.0
Found (μg/L)	1020.0	305.0	1190.2	1090.0	12.5	19.7	5.6
REC[b] (%)	102	101.7	99.2	109.0	104.2	98.5	112.0
RE[c] (%)	2.0	1.7	−0.8	9.0	4.2	−1.5	12.0
RSD[d] (%)	4.0	3.8	1.0	2.3	3.4	1.8	4.3
Inter-day studies[e]							
Added (μg/L)	1000.0	300.0	1200.0	1000.0	12.0	20.0	5.0
Found (μg/L)	1015.0	298.0	1210.0	1100.0	12.3	20.4	5.3
REC (%)	101.5	99.3	100.8	110.0	102.5	102.0	106.0
RE (%)	1.5	−0.7	0.8	10.0	2.5	2.0	5.7
RSD (%)	4.3	2.9	3.7	2.8	1.7	2.5	3.8

REC (%) = $[metal]_{found}/[metal]_{added} \times 100$.
RE (%) = $([metal]_{found} - [metal]_{added})/[metal]_{added} \times 100$.
RSD (%) = $\sigma/[metal]_{mean\ found} \times 100$.
[a] Average of three measurements, three times along a day.
[b] REC, recovery.
[c] RE, relative error.
[d] RSD, relative standard deviation.
[e] Average of three measurements over a week.

Table 4
Mineral contents in bottled waters.

Brand still	Sample No.	Macrominerals, mg/L (%)				Microminerals, μg/L (%)		
		Ca	Mg	K	Na	Fe	Zn	Cu
A	1	9.1 ± 0.5	3.4 ± 1.0	117.5 ± 0.1	209.1 ± 0.4	18.9 ± 3.4	24.7 ± 5.7	13.3 ± 8.5
	2	8.2 ± 0.3	4.0 ± 0.8	107.5 ± 0.5	190.1 ± 2.1	16.5 ± 11.6	16.3 ± 11.0	12.5 ± 0.6
	3	8.4 ± 0.5	4.4 ± 2.1	113.7 ± 0.9	151.0 ± 8.6	42.6 ± 3.9	15.3 ± 3.9	12.3 ± 5.8
	4	0.8 ± 3.0	1.5 ± 4.0	1.0 ± 0.6	6.7 ± 0.4	5.1 ± 3.2	11.9 ± 8.4	9.1 ± 7.0
B	5	212.8 ± 0.8	0.2 ± 1.2	65.7 ± 0.7	46.1 ± 10.2	108.4 ± 10.2	8.3 ± 0.7	0.7 ± 5.7
	6	3.1 ± 0.7	0.3 ± 2.8	137.4 ± 0.4	57.7 ± 6.9	262.9 ± 4.3	7.8 ± 1.3	2.3 ± 1.8
	7	1.5 ± 1.4	0.2 ± 1.7	0.5 ± 0.5	4.2 ± 2.2	2.3 ± 15.2	8.6 ± 0.3	–
C	8	1.6 ± 0.5	28.0 ± 2.6	111.8 ± 0.1	16.6 ± 7.3	83.7 ± 2.0	29.4 ± 2.6	1.2 ± 4.1
	9	238.8 ± 6.7	33.2 ± 3.3	105.7 ± 0.2	16.8 ± 2.4	87.0 ± 13.9	28.5 ± 2.8	2.3 ± 2.6
	10	2.4 ± 1.4	0.4 ± 0.5	94.3 ± 1.2	10.6 ± 5.3	19.5 ± 7.7	30.9 ± 2.8	0.5 ± 0.6
	11	0.2 ± 0.3	0.4 ± 1.3	2.8 ± 1.0	8.1 ± 0.6	1.8 ± 14.2	22.5 ± 4.4	–
D	12	69.7 ± 0.6	12.2 ± 0.5	21.5 ± 0.9	35.4 ± 1.3	2.9 ± 33.6	5.8 ± 17.2	–
	13	55.1 ± 0.5	10.7 ± 0.8	17.3 ± 0.8	31.9 ± 1.0	4.1 ± 6.9	6.9 ± 1.7	–
	14	53.3 ± 1.4	14.3 ± 1.4	15.9 ± 1.6	31.9 ± 2.3	–	9.2 ± 0.6	–
	15	69.9 ± 2.9	20.1 ± 1.6	1.1 ± 1.3	4.8 ± 1.3	–	6.5 ± 21.5	–
Sparkling								
E	16	1.1 ± 2.9	2.1 ± 2.2	246.9 ± 1.0	619.4 ± 1.1	6.3 ± 14.4	11.1 ± 7.2	9.0 ± 0.9
	17	2.3 ± 0.7	2.3 ± 4.2	86.26 ± 3.0	245.2 ± 0.8	13.9 ± 12.7	20.4 ± 13.2	7.9 ± 1.9
	18	1.1 ± 1.3	2.2 ± 4.2	92.7 ± 0.6	221.7 ± 2.3	35.4 ± 11.8	12.8 ± 10.9	9.6 ± 2.2
	19	1.3 ± 1.6	2.2 ± 3.9	96.2 ± 0.2	290.9 ± 0.8	8.9 ± 1.0	13.4 ± 14.9	9.4 ± 1.2
	20	0.2 ± 5.2	1.7 ± 1.7	0.5 ± 0.5	8.2 ± 1.8	83.6 ± 2.5	31.2 ± 10.6	8.7 ± 0.3
F	21	82.5 ± 0.6	20.4 ± 1.5	36.7 ± 0.5	655.6 ± 1.9	29.2 ± 7.3	12.2 ± 11.5	–
	22	82.5 ± 1.4	19.4 ± 2.7	31.8 ± 2.0	618.2 ± 1.5	122.8 ± 1.5	8.3 ± 16.9	1.5 ± 6.9
	23	79.5 ± 0.6	20.2 ± 1.2	41.9 ± 1.1	641.6 ± 2.4	9.4 ± 6.4	12.4 ± 25.8	1.4 ± 3.5
	24	77.8 ± 1.6	21.3 ± 1.3	25.8 ± 1.1	496.6 ± 2.3	196.8 ± 11.7	10.3 ± 18.4	5.1 ± 3.2
	25	89.6 ± 1.2	21.4 ± 2.6	41.8 ± 0.2	667.3 ± 1.8	27.7 ± 3.2	65.9 ± 2.1	1.5 ± 3.1
	26	87.8 ± 0.6	20.0 ± 4.0	42.7 ± 1.5	560.1 ± 3.6	4.5 ± 11.5	4.8 ± 25.0	–
G	27	110.8 ± 1.7	15.0 ± 0.5	122.4 ± 1.8	56.9 ± 1.2	20.3 ± 6.4	15.4 ± 4.5	–
	28	108.4 ± 0.2	14.4 ± 1.1	6.2 ± 0.5	43.4 ± 0.7	49.2 ± 4.4	6.8 ± 1.5	0.7 ± 2.7
	29	112.1 ± 0.4	23.8 ± 0.9	88.7 ± 2.3	45.7 ± 1.4	67.0 ± 4.0	27.6 ± 15.6	2.0 ± 4.3
	30	98.8 ± 1.5	25.9 ± 5.5	78.6 ± 0.2	47.1 ± 1.8	41.0 ± 0.5	5.6 ± 3.6	–
	31	108.0 ± 1.1	33.7 ± 7.3	2.2 ± 0.5	43.1 ± 2.4	58.0 ± 4.3	2.8 ± 0.1	–
H	32	58.2 ± 0.3	10.8 ± 12.3	37.1 ± 1.0	603.0 ± 0.9	35.1 ± 5.6	11.4 ± 14.0	–
	33	81.6 ± 1.8	10.9 ± 2.0	24.8 ± 2.2	596.8 ± 0.5	25.8 ± 2.2	11.2 ± 21.4	–
I	34	123.0 ± 1.6	28.7 ± 21.7	36.2 ± 4.5	671.3 ± 0.1	39.7 ± 2.5	3.0 ± 0.3	–
	35	118.6 ± 0.2	30.1 ± 11.4	39.6 ± 0.4	593.2 ± 1.9	104.4 ± 0.8	6.4 ± 10.9	–
	36	127.4 ± 1.1	29.2 ± 3.2	34.5 ± 1.3	555.1 ± 1.3	36.4 ± 2.8	8.3 ± 1.1	0.9 ± 5.3
	37	123.0 ± 1.5	24.9 ± 5.2	38.3 ± 0.6	535.1 ± 1.2	8.7 ± 1.2	3.5 ± 0.8	–
J	38	3.6 ± 3.6	1.3 ± 1.2	69.2 ± 0.8	359.7 ± 1.4	10.9 ± 2.1	21.7 ± 3.0	7.2 ± 0.7
	39	1.1 ± 1.4	0.2 ± 2.6	0.37 ± 1.0	3.7 ± 1.6	20.7 ± 6.3	24.9 ± 1.4	1.0 ± 4.6

– Not detected.

It is important to stress that the samples with high total dissolved solids (the integrated measure of the concentrations of common ions, Na, K, Ca, Mg, Cl) have a high contribution of Na, with contents ranging 600 mg/L. This fact reinforces the care needed in the consumption of this foodstuff, with special attention to children and adults with renal disorders. Nevertheless, chloride contents are not very high, decreasing the possible influence in the development or contribution to hypertension.

In what concerns microminerals, several natural water samples have important levels of Fe; it is the case of samples 20 and 31 (83.6 and 58.0 μg/L) as well as samples 33 and 39 (25.8 and 20.7 μg/L). Samples 4 and 20 presented 9 μg/L of Cu and sample 39 had 1 μg/L. Only these three natural water samples presented detectable Cu values.

From Table 2, it was possible to obtain more information, namely about the added ingredients in the flavoured waters. Amongst them can be cited:

- fibres, that are listed in 11 samples from brands A, C and F;
- fruit juices or concentrates in about 50% of the samples. Only flavoured brands A, D and G do not refer the addition of this type of ingredient;
- vitamins: 11 samples refer the presence of vitamins of B complex (seven samples) and C (four samples). According to the label, the added amounts are very different in several brands, some of them only refer its presence;
- other bioactive compounds, namely ginseng, L-carnitine, white and green tea and ginkgo biloba; they are present in some flavoured waters from different brands.

Inevitably, these waters also need other ingredients, without positive relation with well-being and health, but necessary to assure the desired quality for the producer and consumers, and the safety of the product, such as, acidifying agents, sweeteners and preservatives.

About 50% of the flavoured samples contained sweeteners as ingredients. There are samples with only one (acesulfame-K, sucralose or aspartame) and with blends of two sweeteners (acesulfame-K and aspartame; acesulfame-K and sucralose). The most used were acesulfame-K (present in 14 samples) and aspartame in 10 samples. It is interesting to note that, in general, the samples from the same brand have the same sweetener, with exception of brand I that use different sweeteners for different flavours.

Brands C and F do not use sweeteners, providing more energetic products (9–13 and 19 kcal/100 mL), respectively. In the case of sweetened samples its energy value ranged from 0.4 to 4 kcal/100 mL.

In what concerns to preservatives and the information contained in the label, each sample can contain one (potassium sorbate or sodium benzoate) or two preservatives (potassium sorbate and sodium benzoate; potassium sorbate and dimethyl dicarbonate; sodium benzoate and dimethyl dicarbonate) simultaneously.

Flavoured waters would not ideally replace natural water, but can be an interesting alternative to soft drinks which are products with more ingredients with negative influence in health, namely obesity and generally in children health. Its moderate consumption can be made with pleasure and without major concerns. It is also important to refer that flavoured waters are more expensive (20–40%) than natural ones.

Confirming the referred above, except for Fe ($p = 0.215$), there were statistical differences in mineral levels between natural waters (with and without natural gas or added gas) ($p < 0.05$ for Ca, Mg, K, Na, Zn and Cu).

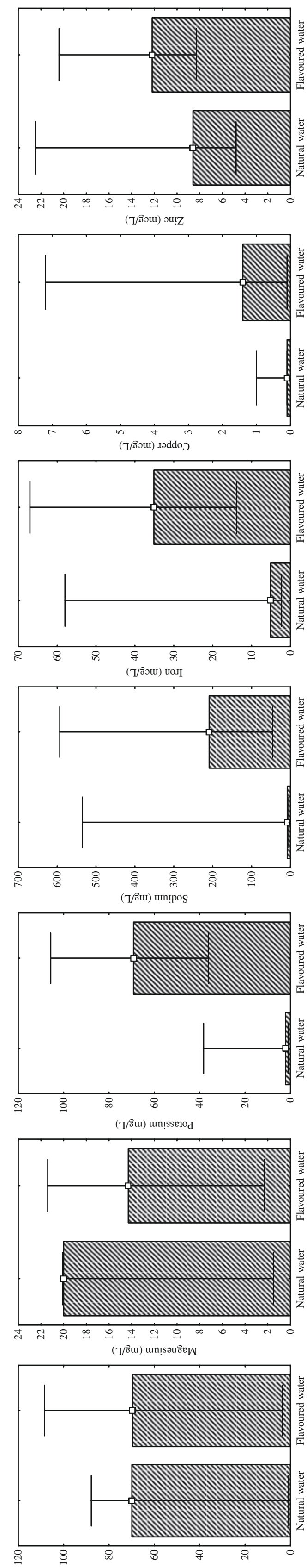

Fig. 1. Concentrations of Ca ($p = 0.767$), Mg ($p = 0.456$), K ($p < 0.001$), Na ($p < 0.001$), Fe ($p = 0.045$), Cu ($p < 0.001$) and Zn ($p = 0.114$) in flavoured waters and correspondent natural water. The bars represent the median and the whisker represents the inter quartile range.

Table 5
Statistical analysis of minerals content results obtained.

	Natural water	Flavoured water	*p*
Ca (mg/L)			
Preservatives			
Potassium sorbate + sodium benzoate (*n* = 12)	0.22 (0.22–0.83)	2.25 (1.12–8.17)	0.018*
Potassium sorbate (*n* = 7)	108.00 (0.99–110.25)	103.55 (4.41–116.98)	0.722
Sodium benzoate (*n* = 6)	96.45 (69.90–123.00)	94.17 (54.62–124.09)	0.138
Acidifying agents			
Citric acid + sodium citrate (*n* = 9)	0.83 (0.22–0.95)	3.07 (1.19–8.31)	0.008*
Citric acid + natural flavour (*n* = 8)	87.80 (87.80–123.00)	86.03 (80.24–121.90)	0.063
Citric acid (*n* = 12)	69.90 (0.52–108.00)	84.25 (53.73–111.76)	0.754
Fruit juice			
Yes (*n* = 19)	1.47 (0.22–87.80)	77.75 (2.25–118.60)	0.845
No (*n* = 10)	69.90 (0.83–108.00)	62.40 (8.93–108.95)	0.959
Sweeteners			
Yes (*n* = 20)	35.68 (0.83–108.00)	54.17 (3.21–111.76)	0.494
No (*n* = 9)	87.80 (0.21–87.80)	82.45 (40.06–98.98)	0.678
Mg (mg/L)			
Preservatives			
Potassium sorbate + sodium benzoate (*n* = 12)	1.72 (1.52–1.72)	2.20 (2.06–3.37)	0.019*
Potassium sorbate (*n* = 7)	24.87 (0.65––33.70)	19.41 (6.03–28.52)	0.875
Sodium benzoate (*n* = 6)	22.48 (20.09–24.87)	21.50 (11.82–29.43)	0.345
Acidifying agents			
Citric acid + sodium citrate (*n* = 9)	1.52 (0.88–1.72)	2.20 (1.69–3.08)	0.007*
Citric acid + natural flavour (*n* = 8)	19.99 (19.99–28.87)	21.35 (20.25–29.09)	0.036*
Citric acid (*n* = 12)	20.09 (0.36–33.70)	14.33 (10.69–25.35)	0.272
Fruit juice			
Yes (*n* = 19)	1.72 (0.36–19.99)	19.41 (2.06–27.95)	0.001*
No (*n* = 10)	20.09 (1.52––33.70)	13.25 (4.32–17.20)	0.028*
Sweeteners			
Yes (*n* = 20)	6.32 (1.52–24.87)	7.54 (2.17–21.61)	0.881
No (*n* = 9)	19.99 (0.36–19.99)	20.42 (16.89–24.69)	0.173
K (mg/L)			
Preservatives			
Potassium sorbate + sodium benzoate (*n* = 12)	0.45 (0.45–1.03)	96.18 (86.26–117.50)	0.018*
Potassium sorbate (*n* = 7)	2.51 (2.21–34.96)	83.65 (36.46–113.24)	0.008*
Sodium benzoate (*n* = 6)	19.72 (1.10–38.34)	28.04 (16.98–37.04)	0.249
Acidifying agents			
Citric acid + sodium citrate (*n* = 9)	0.45 (0.45–1.03)	107.47 (89.47–127.45)	0.008*
Citric acid + natural flavour (*n* = 8)	42.74 (38.34–42.74)	36.45 (32.45–41.24)	0.030*
Citric acid (*n* = 12)	2.21 (1.10–2.81)	72.11 (18.39–102.82)	0.002*
Fruit juice			
Yes (*n* = 19)	2.81 (0.45–42.70)	65.65 (36.70–96.18)	0.014*
No (*n* = 10)	1.10 (1.03–2.21)	83.65 (16.98–114.68)	0.005*
Sweeteners			
Yes (*n* = 20)	1.07 (0.45–2.21)	82.42 (36.42–112.17)	<0.001*
No (*n* = 9)	42.74 (2.81–42.74)	41.79 (28.77–99.99)	0.594
Na (mg/L)			
Preservatives			
Potassium sorbate + sodium benzoate (*n* = 12)	8.20 (6.70–8.20)	245.20 (209.00––359.70)	0.017*
Potassium sorbate (*n* = 7)	43.10 (8.10–535.10)	57.30 (43.98–583.68)	0.002*
Sodium benzoate (*n* = 6)	269.95 (4.80–535.10)	295.25 (31.90–612.73)	0.029*
Acidifying agents			
Citric acid + sodium citrate (*n* = 9)	6.70 (5.45–8.20)	221.70 (170.55–325.30)	0.007*
Citric acid + natural flavour (*n* = 8)	560.10 (535.10–560.10)	629.90 (564.63–664.38)	0.050*
Citric acid (*n* = 12)	8.10 (4.80–43.10)	39.40 (20.58–46.85)	0.002*
Fruit juice			
Yes (*n* = 19)	8.20 (8.10–560.10)	496.60 (57.70–619.40)	0.001*
No (*n* = 10)	6.70 (4.80–43.10)	46.40 (34.53–160.76)	0.005*
Sweeteners			
Yes (*n* = 20)	8.20 (4.80–43.10)	199.60 (46.35–506.25)	<0.001*
No (*n* = 9)	560.10 (8.1–560.10)	496.60 (16.7–648.60)	0.051
Fe (µg/L)			
Preservatives			
Potassium sorbate + sodium benzoate (*n* = 12)	83.60 (5.10–83.60)	13.90 (8.90–18.90)	0.128
Potassium sorbate (*n* = 7)	8.60 (3.00–58.00)	41.80 (35.43–79.45)	0.050*
Sodium benzoate (*n* = 6)	4.70 (0.81–8.60)	20.25 (2.30–55.85)	0.046*
Acidifying agents			
Citric acid + sodium citrate (*n* = 9)	20.70 (5.10–83.60)	16.50 (9.90–39.00)	0.594
Citric acid + natural flavour (*n* = 8)	4.50 (4.50–8.60)	38.05 (28.00–118.18)	0.012*
Citric acid (*n* = 12)	2.05 (1.05–58.00)	38.05 (7.95–79.45)	0.158
Fruit juice			
Yes (*n* = 19)	4.50 (2.30–25.80)	35.40 (13.90–104.30)	0.044*
No (*n* = 10)	5.10 (0.80–58.00)	19.60 (3.83–44.25)	0.646

(*continued on next page*)

Table 5 (*continued*)

	Natural water	Flavoured water	*p*
Sweeteners			
Yes ($n = 20$)	8.60 (3.00–58.00)	27.70 (9.40–42.20)	0.601
No ($n = 9$)	4.50 (1.80–4.50)	49.2 (23.55–104.90)	0.015*
Zn (μg/L)			
Preservatives			
Potassium sorbate + sodium benzoate ($n = 12$)	31.20 (11.90–31.20)	16.30 (13.80–21.70)	0.176
Potassium sorbate ($n = 7$)	7.35 (2.80–11.73)	9.85 (6.50–24.55)	0.015*
Sodium benzoate ($n = 6$)	6.65 (5.10–8.23)	6.65 (5.00–8.79)	0.991
Acidifying agents			
Citric acid + sodium citrate ($n = 9$)	24.90 (11.90–31.20)	15.30 (11.95–21.05)	0.26
Citric acid + natural flavour ($n = 8$)	4.80 (4.80–6.00)	9.30 (12.35–6.88)	0.043*
Citric acid ($n = 12$)	7.75 (2.80–19.68)	10.30 (6.83–28.28)	0.015*
Fruit juice			
Yes ($n = 19$)	8.60 (4.80–24.90)	12.20 (8.30–21.70)	0.796
No ($n = 10$)	6.35 (2.80–11.90)	12.25 (6.55–18.40)	0.018*
Sweeteners			
Yes ($n = 20$)	8.90 (5.95–21.65)	11.25 (7.13–16.08)	0.925
No ($n = 9$)	4.80 (4.80–22.50)	12.40 (23.55–30.15)	0.008*
Cu (μg/L)			
Preservatives			
Potassium sorbate + sodium benzoate ($n = 12$)	8.65 (8.65–9.08)	9.35 (7.95–12.45)	0.051
Potassium sorbate ($n = 7$)	0.10 (0.07–0.15)	0.61 (0.10–1.80)	0.018*
Sodium benzoate ($n = 6$)	0.08 (0.06–0.10)	0.10 (0.09–0.29)	0.285
Acidifying agents			
Citric acid + sodium citrate ($n = 9$)	8.65 (4.85–8.08)	9.35 (7.56–12.35)	0.018*
Citric acid + natural flavour ($n = 8$)	6.10 (0.06–0.10)	1.13 (0.10–1.51)	0.026*
Citric acid ($n = 12$)	0.10 (0.10–0.15)	0.33 (0.10–1.07)	0.028*
Fruit juice			
Yes ($n = 19$)	0.10 (0.10–1.04)	1.46 (0.50–7.17)	0.001*
No ($n = 10$)	0.15 (0.10–9.08)	0.41 (0.10–12.30)	0.063
Sweeteners			
Yes ($n = 20$)	0.15 (0.10–8.65)	1.43 (0.10–9.27)	0.005*
No ($n = 9$)	0.10 (0.10–0.10)	1.39 (0.61–1.93)	0.012*

* Statistically significant $p < 0.05$. Data are presented was median (1st quartile–3rd quartile).

After the presented discussion it is consensual that the minerals contents in flavoured waters are higher than in natural ones. One justification for that is the use of several ingredients in the salt form.

3.2.1. Flavour factor analysis

Considering only the flavour factor, the contents of K, Na, Fe and Cu are higher in the flavoured waters than in the natural ones. For Mg and Ca median concentrations were slightly higher in the natural waters. However, the difference observed between the median concentration of the two groups (natural and flavoured) are statistically significant only for K, Na, Cu ($p < 0.001$) and Fe ($p = 0.045$). Fig. 1 shows the median concentration of the minerals studied in flavoured and natural waters.

3.3. Individual mineral composition

Calcium is the most important macromineral, with amounts ranging from 0.2 to 213 mg/L. Analysing Table 4 it was possible to verify that Ca contents are higher in sparkling waters (except in brands E and J, that have added gas) than in still waters. In the former group included the samples with the highest content in total dissolved solids and the one with the lowest contents (brand J). In flavoured still waters, Ca levels increased, except in brand D. This brand only has preservatives, sweeteners and acidifying agents.

Samples 5 and 9 presented the highest Ca contents (140 and 600 times higher than the corresponding natural water). In this case, the addition of calcium lactate (Table 2) as acidifying agent can justify the increased contents. Nevertheless the detected values are lower than the claimed in the label (Ca 1200).

In sparkling waters Ca levels were kept fairly constant in all samples. As an exception, in brand H, the flavoured water had lower contents than the natural one. This situation could not be explained with the available information in Table 2.

Magnesium is a cofactor in almost all phosphorylation reactions involving ATP and is an indirect antioxidant, being important for the control of the pro-oxidant and antioxidant status (Lukaski, 2004). Its concentration ranged from 0.2 to 33.2 and 0.2 to 33.7 mg/L in still and sparkling waters, respectively. Sample 9 had the highest Mg contents (when compared with the corresponding natural sample). This increment was higher than the determined in sample 8 with magnesium carbonate incorporation. According to Table 2 this sample should contain 450 mg/L. This claim does not correspond to the actual water content, and therefore, the consumer is misled when looking for a good source of Mg. Mg contents have little variation in the water samples evaluated. There are samples with an increase in the contents and in others a decrease occurs.

Potassium is the most abundant positively charged electrolyte inside cells, being very important for the muscle contractility, including cardiac muscle (European Food Safety Authority (EFSA), 2006).

K concentration ranged from 0.5 to 137.4 mg/L and 0.4 to 246.9 mg/L in still and sparkling waters, respectively. In general, water samples (still and sparkling) presented an increment in K levels in the flavoured waters that can be explained by the addition of ingredients in a K salt form, which is the case of some preservatives.

Sodium is the major extracellular electrolyte with functions in nerve conduction, active transport and formation of the mineral apatite of the bone (WHO/FAO, 2003).

Table 4 points out high Na contents in some water samples, as referred above. In flavoured waters, both still and sparkling, a significant increase in the Na contents was verified, comparing to the natural ones. One of the major influential factors is the addition of sodium benzoate and sodium citrate as preservatives.

Iron is essential for the haemoglobin (oxygen transport), myoglobin, fatty acid, DNA and neurotransmitters synthesis, in peroxide conversions, in purine metabolism and in the nitric oxide production (Lukaski, 2004).

Fe contents of samples 14 and 15 (Table 4) were lower than the LOD value. Samples 4, 7, 11, 12, 13 and 26 (Table 4) presented levels between LOD and LOQ values.

In the other samples, Fe contents ranged from 16.5 to 262.9 μg/L and 6.3 to 196.8 μg/L in still and sparkling water, respectively. In samples with added gas, iron contents are lower than in the natural ones, which is an interesting factor.

Zinc is essential to enzymes function, acting as catalyst or stabilising protein structure (Silvera & Rohan, 2007; Zuliani, Kralj, Stibilj, & Milačič, 2005). Zn in excess competes with the absorption of Cu and Fe. Zn was detected in all samples, in levels ranging from 5.8 to 30.9 μg/L and 2.8 to 65.9 μg/L in still and sparkling waters, respectively. The behaviour presented is not constant, with increased levels in some samples and decreased in others, comparing flavoured and natural waters. Sample 25 (melon/mint) presented the highest contents of Zn and sample 10 (pineapple/fibre) the second higher content. A possible justification can be the flavour used in the case of sample 25 (brand F). In brand C all samples had similar contents regardless of flavour contribution.

Copper is an essential cofactor for a variety of enzymes and, like Zn and Fe, is involved in the regulation of the expression of the genes for the metal-binding proteins (Zuliani et al., 2005). Deficient intakes can promote breast cancer and cardiovascular diseases. Cu was not detected in all samples of brands D and H, neither in some natural water (samples 7, 11, 26, 31 and 37). As shown in Table 4, the presence of flavours can increase Cu levels in water samples. In brand I, sample 36 (strawberry flavour) is the only one with a detectable Cu level. In brand G only samples 28 and 29 (lime and apple) had a detectable Cu level.

3.4. Effects of same labelled compounds in mineral composition

Table 5 shows the results of the statistical analysis considering the following factors: preservatives, acidifying agents, fruit juice and sweeteners. These ingredients are added to natural waters and this study aimed to verify its influence in the contents of the macro and microminerals evaluated. Their influence in each mineral will be appreciated individually.

3.4.1. Calcium

Taking into account the preservatives added, it was observed that the blend of potassium sorbate and sodium benzoate lead to significant statistical differences ($p = 0.018$). For the remaining preservatives the differences observed are not significant ($p > 0.05$).

In what concerns acidifying agents, only the waters with, simultaneously, citric acid and sodium citrate presented statistically significant differences from its natural corresponding water ($p = 0.008$). The addition of fruit juice ($p = 0.845$) as well as sweeteners does not influence Ca concentration.

3.4.2. Magnesium

In the case of Mg the statistical study showed that the addition of preservatives (potassium sorbate and sodium benzoate) ($p = 0.019$) and acidifying agents increased significantly Mg concentration. Regarding fruit juice, the statistical values are $p = 0.001$ and 0.028 for flavoured waters with and without fruit juice, respectively.

No other factor affected Mg concentration in a significant way.

3.4.3. Potassium

The addition of preservatives, only one (potassium sorbate) or in combination (potassium sorbate and sodium benzoate) as well as all acidifying agents, increased significantly K levels ($p = 0.008$ and 0.018, respectively) (Table 5).

Regarding the presence or absence of juice, there is also a significant statistical difference in K concentration between flavoured and natural waters. The addition of sweeteners also increased significantly K concentration ($p < 0.001$).

3.4.4. Sodium

Statistical analysis showed that the influence of numerous factors led to significant differences in the results. This can be seen in Table 5.

3.4.5. Iron

The presence of potassium sorbate or sodium benzoate, as preservatives, induced significant differences in Fe contents, as well as the presence of citric acid and natural flavours. Also, significant differences, in Fe content, can be verified amongst waters without fruit juice or sweeteners.

3.4.6. Zinc

The statistical analysis for the influence in Zn concentration showed that the major influence came from potassium sorbate and citric acid (Table 5). As referred for Fe, significant differences amongst waters without fruit juice and sweeteners could also be noticed.

3.4.7. Copper

Regarding the influence of the considered factors in Cu concentration, the ones that caused statistical significant differences were: potassium sorbate (in the preservative group), all acidifying agents and the presence of fruit juice. The presence of sweeteners influenced the levels of this micromineral in all samples.

4. Conclusion

This study leads to conclude that flavoured waters can be an adequate alternative to consumers that do not like natural water. The different ingredients added to natural waters hardly influence its mineral composition. All consumers are advised to read the label content, in order to avoid some health problems that can occur with some mineral waters and some specific groups of consumers. Also, flavoured waters could represent advantages due to the presence of certain minerals, some vitamins, antioxidants and bioactive compounds. Some preservatives, acidifying agents and sweeteners are not hazardous if consumed with moderation.

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